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project

Sustainable productivity improvements in allium and solanaceous vegetable crops in Indonesia and sub-tropical Australia.

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Abbreviations

ACIAR	Australian Centre for International Agricultural Research
asl	Above sea level
AUD	Australian dollar
AVRDC	Asian Vegetable Research and Development Centre (now World Vegetable Centre)
BAP	Benzyl amino purine
BPTP	Balai Pengkajian Teknologi Pertanian
ChiVMV	Chili veinal mottle virus
CMV	Cucumber mosaic virus
DAF	Queensland Department of Agriculture and Fisheries
DAI	Days after inoculation
DM%	Dry Matter concentration
FFS	Farmer Field School
GCLV	Garlic common latent virus
GLV	Garlic latent virus
GVA	Garlic virus A
GVB	Garlic virus B
GVC	Garlic virus C
GVD	Garlic virus D

GVE	Garlic virus E
GVX	Garlic virus X
IBA	Indole Butyric Acid
ICM	Integrated crop managment
IPB	Bogor Agricultural University
IVEGRI	Indonesian Vegetable Research Institute
M&E	Monitoring and Evaluation
MOA	Ministry of Agriculture
MS	Murashige and Skoog media
NAA	Naphthaleneacetic acid
NSW	New South Wales (Australia)
OYDV	Onion yellow dwarf virus
PCR	Polymerase Chain Reaction
PepMV	Pepper mottle virus
PVY	Potato virus Y
Qld	Queensland (Australia)
RT-PCR	Reverse transcription-polymerase chain reaction
SA	South Australia (Australia)
SCM	Supply Chain management
ShVX	Shallot virus X
SLV	Shallot latent virus
SWOT	Strengths, weaknesses, opportunities, threats
SYSV	Shallot yellow stripe virus
TC	tissue culture
TMV	Tobacco mosaic virus
ToT	Training of Trainer
UGM	Universitas Gajah Mada
USDA	United States Department of Agriculture
UQ	University of Queensland
VC	Value Chain
Vic	Victoria (Australia)
WTO	World Trade Organization

2 Executive summary

Shallot and chilli represent the two most important vegetable crops in Indonesia and are essential in defining Indonesia's unique cuisine. About 1 million tonnes of shallots are consumed in Indonesia per year and average crop yields are about half the maximum obtainable yield. The allium crop group, predominantly shallot and garlic, represent more than 90% of Indonesian vegetable imports. The allium crops are constrained by a range of factors that substantially reduce yield including seed quality, soil fertility, and pests and pathogens. Project SMCN/2009/056 had a strong focus on these with the aim of increasing the productivity of shallot and chilli farming systems.

A benchmarking of agronomic practices in shallot and chilli farming was conducted with about 50 farmers in each of the focus regions of Brebes, Cirebon, Nganjuk and Bantul-Yogyakarta. These studies developed and documented critical information on current farmer practices on varieties, seed supply, fertiliser and pesticide use, and market chains, for shallot seed supply and fresh product consumption. The benchmarking survey identified soil fertility, crop nutrition and pest management as the most significant issues for farmers and that the over-application of fertilizer and pesticide inputs is common.

The issue of seed quality was identified as a major constraint to shallot productivity in Indonesia and garlic in Australia. Since shallot and garlic are vegetatively propagated, the project has had a considerable focus on the identification of allium (shallot and garlic) virus complexes in Indonesia and Australia. However, the regional distribution of viruses in Indonesian shallot production, the virus species present and the level of infection were not known, hence the potential impact of viruses on shallot crop productivity was unknown at the initiation of the project. To assess this issue, the project developed world-first diagnostics for allium viruses and provided training for the Indonesian project team. This improvement in allium virus diagnostics has been a major achievement for the project providing molecular probes to differentiate individual virus species within each of the three genera (Potyvirus, Carlavirus and Allexivirus). The results for shallot in Indonesia showed high infection levels of potyvirus and allexivirus. In Queensland, more than 98% of collected samples tested positive for the presence of the three virus groups. The surveys identified a number of virus species not previously known to be present in alliums in Indonesia and Australia.

A comparison of virus infection from bulbs collected from commercial shallot crops in Brebes and Cirebon with bulbs derived from true-seed shallot (TSS) showed high virus infection in field bulbs compared with plants grown directly from TSS. The Indonesian and Australian teams collectively evaluated a range of tissue culturing techniques (including chemotherapy, thermotherapy and electrotherapy) to remove the virus from shallot and garlic explants. Small explants (1mm) combined with chemotherapy were effective in eliminating viruses from shallot bulbs compared with larger explants (2mm or 3mm). True-shallot seed (TSS) is also an important strategy in initiating virus free planting material. However, research in Indonesia on the development of TSS has identified highly variable but low levels of inflorescence formation (14-43%), poor floret pollination rates (12-30%) and poor germination rates (0-1%). In contrast, when grown in Queensland, two of the Indonesian varieties demonstrated prolific inflorescence production, high floret fertilisation and high germination, indicating this activity is suited to subtropical Queensland conditions.

Consistent with the farmer benchmarking surveys, a nutrient budgeting survey of shallot crops identified that farmers apply fertiliser at rates greatly in excess of crop requirement. Nonetheless, there was considerable variability in farmer fertiliser application rates where a small number of farmers still under-apply fertiliser. Nitrogen fertiliser is applied at a rate of about 2-3 times total crop requirement, P at about 5 times crop requirement

and K at about 2-3 times crop requirement. Research on garlic in Australia showed farmers tend to under-apply N fertiliser and crop uptake to maximise yield is in the order of about 200 kg N ha^{-1}. Further trials in Australia evaluated the reselection of improved garlic lines (cv. Glenlarge) and, which combined with nutrition and seed storage, has lifted garlic yield from about 6 t ha^{-1} at the start of the project to about 17 t ha^{-1} at the end of the 2018 season.

In shallot, Indonesian research identified a complex of three different fusarium species which were found in association with moler disease, including *F. solani*, *F. accutatum* and *F. oxysporum.* The combination of these species resulted in the expression of the moler symptoms including, chlorosis, leaf-twisting and wilting. Moler disease of shallot is more prevalent in the wet season (30-60% incidence) compared with dry season (≈10%) production. This finding will provide a better understanding of the disease and an improved resistance screening procedure.

Field surveying for chilli viruses was conducted across Indonesia and identified a suite of viruses affecting solanaceous crops. Begomoviruses and predominantly Pepper yellow leaf curl virus (PYLCV) was the most common virus identified in Indonesia. The PYLCV was identified as endemic across all of Indonesia, including Eastern Indonesia in close proximity to Australia.

The development of begomovirus resistant chilli germplasm was a key project objective. A suite of about 111 accessions from Indonesian, Australian and AVRDC germplasm collections were screened for resistance to PYLCV. In early assessments, lines IPBC13 and 19, the commercial cultivar Kopay and accession C12 appeared immune (0% symptoms) and IPB cultivars Annis (4%), Yuni (6%) and IPBC12 (2%) were resistant. These lines represent the best material for developing further resistance in chili and bell capsicum breeding lines. DAF accession 69-3 expressed delayed symptom development (10 weeks after inoculation) suggesting a different tolerance mechanism is operating which, in combination with C12, could provide more durable genetic resistance. A series of field research trials on improvements in chilli root system performance identified about 5 wild accessions of *Capsicum chinense* that have high performing root systems and may be used to improve nutrient use efficiency in capsicum.

A strong focus of the project has been the building of capacity at multiple levels, including support for professional and farmer training and postgraduate study opportunities.

3 Background

The Indonesian Government, through the Directorate General of Horticulture (DGH), identified a priority to expand the production area of the premier vegetable commodities potato, chilli and shallot due to the high market demand and economic value of these commodities. In domestic Indonesian vegetable production, chilli and alliums (particularly shallots) represent the most significant vegetables in terms of value and tonnage (Table 1) but yield of these is very low. This project developed themes based on the outcomes of the ACIAR chilli project HORT/2004/048 and a scoping study (Harper et al. 2010) that evaluated the research needs of lowland-coastal shallot-chilli production systems. A key focus of this project was to identify and address crop productivity constraints in shallot and chilli production in 4 key production regions of Java (Brebes, Cirebon, Bantul and Nganjuk).

About 1 million tonnes of shallots are consumed in Indonesia per year. Domestic production is around 150,000 ha with a highly variable yield of about 4-18 t ha^{-1} of which some 20% is saved for replanting (seed-bulbs). The domestic production of alliums, and especially garlic, has continued to decline in both value and production. This loss in industry value has been made up through import substitution with a higher quality product from China. The allium crop group (comprising shallot, garlic, leeks and onions) represents 92% of all vegetable imports into Indonesia at a substantial cost of US$72.8 million. The potential to increase domestic productivity of these commodities can greatly offset this import cost. This issue was specifically identified as a priority by Indonesian government ICHORD representatives at the project development meeting in Jakarta December 2010.

Table 1. Production and import data for key vegetables in Indonesia in 2004.

Commodity	Domestic production			Imports		
	Tonnage	Value ($US million)	Value (% total)	Tonnage	Value ($US million)	Value (% total)
Shallot	757,399	603	12.1	48,927	14.2	18
Chilli	714,705	1,339	26.8	nil	nil	nil
Leeks and other alliums	N/A	378	7.6	N/A	5.3	6
Garlic	28,851	22.9	0.5	243,720	53.3	68

Source: Horticultural producers and supermarket development in Indonesia. World Bank 2007.

For both shallot and chilli there is a further significant market issue that peak demand for these commodities is associated with cultural events that do not coincide with optimal crop productivity. The development of cropping strategies to target key limited production windows, such as in the wet season, could deliver yield gains.

The key issues addressed above are also of direct relevance to allium and solanaceous vegetable production in Australia. In Australia, Queensland is the most significant producer of capsicums (bell peppers) with most (60-70%) of this being in the coastal

catchments that drain to the Great Barrier Reef Lagoon and Moreton Bay. As such, the issue of improved nitrogen management is critical. Plant pathogens endemic in Indonesia present a potential biosecurity risk to Australian solanaceous crop production and an improved understanding of this can will allow better preparation for disease incursions. Viruses in allium crops, particularly garlic, has seriously limited Australian garlic production and the sourcing of clean planting material has been an ongoing issue in the redevelopment of profitable garlic production in Australia.

The project concept identified a need to improve the overall agronomy of shallot, garlic and chilli crops in Indonesia and capsicum and garlic crops in Australia to deliver increased crop productivity and quality. In achieving this, the following research questions were addressed.

(1) What is the status of shallot/chilli crop and marketing systems in Indonesia?

The status of current crop cultural and agronomic practices and a crop supply chain analysis needed to be determined and understood to provide objective data from which productivity improvements can be assessed.

(2) What is the spectrum and incidence of major allium and chilli diseases and what effective strategies can be evaluated and developed to improve disease management?

At the projects initiation the status of allium (shallot and garlic) and chilli/capsicum diseases were not well defined in Indonesia and Australia. Previous data on disease incidence in alliums in both Indonesia and Australia was incomplete and crop losses attributable to pathogens were not quantified. Dutch research by Plant Research International Wageningen (2006) highlighted a significant incidence of Onion yellow dwarf virus (OYDV) and Shallot yellow stripe virus in shallot. Similarly, seed-borne viruses in chilli were also identified including Tobacco mosaic virus and Cucumber mosaic virus. There was high incidence of OYDV in the large shallot cultivation areas around Cirebon, Brebes and Tegal, but incidences were relatively low in other areas. In Australia, the expression of virus symptoms in garlic crops was widely recognised and perceived to reduce crop productivity, which resulted in a substantial decline in garlic production in Australia. In Queensland, the virus disease complex and the impact of viruses on crop productivity through infected planting material was not known. In Indonesia, the decline in garlic production has been even more substantial and product importation has increased greatly such that garlic is the single biggest imported vegetable commodity. AVRDC has shown yield reductions of up to 59% in naturally infected garlic varieties compared with the yield of virus-eradicated planting material (AVRDC, 2003). Hence it was speculated that the reduction in shallot and garlic production in Indonesia would at least, in part, be a function of poor, virus infected seed-bulbs as has been demonstrated in Australia. At a project development meeting held in December 2010 in Jakarta the ICHORD representative highlighted a governmental desire to redevelop garlic production.

(3) Can pathogen tested shallot and garlic seed-bulb be produced, does it infer greater crop productivity and is a system that produces commercially available seed-bulb feasible?

The research question in improving the productivity of shallot and garlic crops is how to develop seed-bulb production systems that can supply clean virus/pathogen checked planting material to farmers. At all levels of government and within industry shallot seed-bulb quality is a major production constraint (Harper et al. 2010). Since shallot and garlic seed are grown from vegetative material kept from previously harvested crops, contamination with pathogens (particularly viruses) increases with each generation often resulting in greatly reduced size, quality, yield and market value of these commodities. More recently in Indonesia, the problem in shallots has been averted to some extent through the use of true (botanical) seed. This has given higher yield and has helped to

partially fill critical low production gaps when bulb-shallot-seed is limited. However, the true seeded shallots (TSS) have a pale colour, low pungency and are relatively large; traits that are not acceptable in the Indonesian domestic market.

(4) Can improvements in integrated crop management be identified and what strategies can be implemented?

The yield of both shallot and chilli has declined (Adiyoga 2009) indicating a need for focussed attention on crop agronomy and disease management. To address this, research was conducted to identify current nutrient use efficiency in shallot chilli rice systems and make recommendations on further research required. Fertiliser represents a substantial cost to small Indonesian farmers and average fertiliser costs for shallot and chilli are 15-30% of the total production cost (including labour). The project developed and evaluated baseline data on nutrient application and removal for allium crops. Fertiliser use efficiency was also a key environmental issue in Queensland where vegetable production is practiced in some regions that drain to the Great Barrier Reef lagoon and the project developed improved genetics for root systems in capsicum and chilli. Overuse of chemicals is a key issue resulting in resistance and excessive cost. The project evaluated best management practices for control of key pathogens in shallot and chilli and reducing pesticide-use. Viruses remain a key limitation in chilli production. The project screened chilli germplasm for geminivirus resistance and further evaluated and extended the chilli ICM strategies developed in the AVRDC/ACIAR project CP/2004/048.

4 Objectives

The overall project aim was to raise the productivity of allium (shallot and garlic) and chilli/capsicum cropping systems through improved agronomy, pathogen and virus management, germplasm and seed improvement, and fertiliser management. The project had a particular focus on shallot cropping in coastal Java as the previous ACIAR-AVRDC project specifically focussed on chilli cropping.

The project was focussed on four key objectives.

Objective 1: To characterise the agronomic practices of shallot-chilli-rice production and supply systems in key areas across Java.

A comprehensive literature review of shallot production and research in Indonesia was completed and a baseline agronomy survey conducted with approximately 50 farmers in each of the districts of Cirebon, Brebes, Bantul/Yogyakarta and Nganjuk. A series of meetings were held in each of the key areas (Cirebon, Brebes, Bantul/Yogyakarta and Nganjuk) throughout the project. A survey of the shallot supply chain stakeholders was also conducted to evaluate shallot market and seed supply dynamics.

Objective 2: To identify and quantify the incidence of significant pathogens and agronomic issues in allium and solanaceous crops in Indonesia and Australia.

Surveys of the major pathogens of shallot and chilli production systems were conducted intensively across the priority areas of Java (Cirebon, Brebes, Bantul/Yogyakarta and Nganjuk) and less intensive surveys across other parts of Indonesia including West Sumatra, West Nusa Tenggara (Lombok and Bima), South Sulawesi, Bali, Papua and West Papua. The most significant pathogens were PYLCV in chilli and virus complexes and fusarium in shallots.

Intense surveys of farmer agronomic practices and pest management practices were completed to provide a position statement and benchmarking of current status.

Objective 3 To develop a system to supply clean pathogen tested shallot (Indonesia) and garlic (Australia) seed and evaluate cultivar performance.

A large range of shallot and garlic and chilli germplasm was assembled in Indonesia and Australia. Germplasm was shared between partner countries. The project aimed to develop a pathogen tested seed scheme in Indonesia and a garlic seed supply program in Australia. Furthermore, improved agronomic practices and True Seed Shallot production were evaluated.

Objective 4 Evaluate, develop and promote improved chilli and shallot agronomy and disease management strategies.

This objective aimed to use a range of strategies to address agronomy and disease constraints to shallot and chilli production. This included genetic screening for disease resistance, evaluation of integrated crop management strategies, assessments of nitrogen responses and nutrient budgets in shallot, and improved root systems for chilli. Farmer workshops and training were conducted to provide best crop management options, market and seed supply issues and the priority issue of improved pest and nutrient management.

5 Methodology

5.1 Objective 1: To characterise the agronomic practices of shallot-chilli-rice production and supply systems in key areas across Java.

Objective 1.1 Literature review

This objective was addressed by initially conducting a thorough review of literature and by collating available data and information on shallot production and agronomic practices in Java. The review considered a wide range of production attributes including, fertiliser input rates, soil fertility, pest and disease incidence, pesticide application, cultivars grown, timing of production, crop yields, crop quality, production windows, marketing channels etc. This also included the identification of any existing “seed” schemes for allium (shallot and garlic) production in Java.

Objective 1.2 Conduct strategic surveys of shallot chilli agronomic, production and marketing practices.

A review of existing studies, reports, and government statistics was carried out at the start of the research process to take stock of current knowledge and available information, to identify data gaps, and to inform the primary data collection process, particularly the choice of fieldwork locations and key informants. Secondary data was further reviewed during the report writing stages to support and illustrate the analysis and findings. A baseline agronomic and production survey was conducted in the 4 key production areas of Java (Cirebon, Brebes, Bantul/Yogyakarta and Nganjuk). The survey included a primary survey with farmers and traders and focused group discussions with selected key informants.

The baseline survey integrated qualitative and quantitative survey methods to meet the study objectives. The primary surveys were carried out through face-to-face interviews with 120 farmers and 60 traders from the 4 focus regions to collect information on the socio-economics of shallot farming, pesticide usage, other inputs used and some marketing information. The primary survey provided detailed information on variables pertaining to individual farmers and traders, which allowed a more rigorous analysis of some important variables and factors. Responses were recorded in a questionnaire form and collected and collated by IVEGRI for data entry, analysis and tabulation.

In each survey location, shallot farmers were identified and from this 30 farmers were randomly selected as survey respondents. In addition, three types of traders (i.e. assembly traders, wholesalers and retailers) were deliberately selected for collecting information on the marketing aspects. For each trader type, five traders were selected for interviewing, giving a total of 15 selected traders. The same selection process and a proportional number of respondents were applied to all study sites.

A structured questionnaire was developed and used for interviewing shallot traders in Cirebon, Brebes, Bantul and Nganjuk. The research team interviewed three categories of traders (local assembly traders, inter-regional assembly traders/wholesale traders and retail traders). These were considered as having the most important role in bridging supply between producers and consumers in the shallot supply chain. A qualitative approach was used to collect information on social and institutional issues involved in shallot farming at each study site. Preliminary information regarding the shallot seed systems were obtained through focus group discussions in each research location with knowledgeable shallot farmers, seed growers, traders and regional agricultural officers. Secondary data related to production and market prices of shallot, and other published

statistics about each research site were collected from national government agencies, and national, provincial, and district agricultural extension and statistic offices.

Key references were used for developing the analytical framework that guided this supply chain study. A selective approach to data collection and the choice of tools and methods for data analysis (SWOT – Strengths, weaknesses, opportunities and threats analysis) was followed taking into consideration the resources and time allocated to each study. The study covered a wide range of issues deemed important for understanding the structure, function and performance of shallot supply chains with an emphasis on the constraints faced by chain actors and the opportunities to improve the supply chain.

5.2 Objective 2: To identify and quantify the incidence of significant pathogens and agronomic issues in allium and solanaceous crops in Indonesia and Australia.

Field surveys of Indonesian shallots and Australian Garlic

A field survey of shallot crops in Indonesia was conducted to evaluate the incidence of both known and potentially unidentified viruses and other pathogens of shallot. The survey was intensively conducted in the target highland and lowland regions of West, Central and East Java. Under the project extension the outer islands of Indonesia including West Sumatra (Bukittinggi), West Nusa Tenggara (Lombok and Bima), South Sulawesi (Enrekang), Bali, Papua and West Papua were also included. A detailed survey methodology was developed to determine the distribution of viruses and training in the survey protocol and pathogen identification was provided to all stakeholders at the Universitas Gadjah Mada. A survey of virus complexes in Australian garlic crops was also conducted in the subtropical and temperate climes.

Over the course of the project, surveys were conducted in Java at Brebes, Cirebon, Nganjuk and Bantul (2012-2013) and subsequently under the project extension from 2016-2017 on several other production regions in Java, West Sumatra, Bali, West Nusa Tenggara, South Sulawesi, Papua and West Papua and (Fig. 5.1). Virus detection was carried out at the Plant Virology Laboratory, Bogor Agricultural University (IPB) and Laboratory of Tropical Horticulture Study Center, IPB.

On average, ten (10) fields were selected in each region; each field was divided into 10 blocks; then 10 samples were collected from each block. Disease incidence in the field was assessed based on symptom observation. Leaf samples were collected from chilli plants for virus detection by using specific antibodies (CMV, ChiVMV, PVY, TMV, and PepMV) and universal primer of *Begomovirus* (SPG1/SPG2) for PYLCV. Further sequence analysis was conducted to confirm PYLCV. For the detection of shallot viruses, bulbs were harvested and taken to the laboratory. Bulbs collected from the field were then grown in the laboratory and leaf samples were taken after 30 days for virus detection conducted by DIBA using specific antibodies to GarCLV, SLV, OYDV, and SYSV. Farmer interviews were also conducted to collect information related to cultural techniques and crop management.

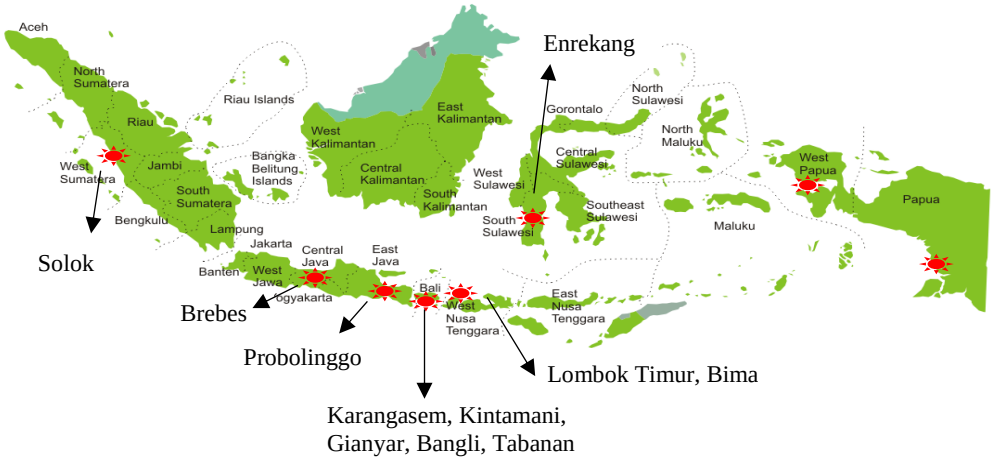


Figure 5.1. Survey locations for mapping distribution of major viruses infecting shallot and chilli pepper.

Methodology developed to identify allium viruses

The development of diagnostics for allium viruses was a key task underpinning the rigour of allium virus surveys in Indonesia and Australia.

Infection of alliums (shallot and garlic) by viruses is common in allium producing regions, including Australia, and substantially reduces crop quality and yield. Viruses commonly detected in garlic include members of the *Poty-*, *Carla-* and *Allexivirus* genera, and a Tospovirus, *Iris yellow spot virus* (IYSV). The proliferation of viruses throughout allium crops occurs primarily through vegetative propagation of infected bulbs. Insect vectors also contribute to virus spread, where potyviruses- and carlaviruses are transmitted by aphids, and allexiviruses by mites. Extensive research has been carried out globally to identify and characterise viruses infecting alliums, particularly garlic.

Multiple viruses are known to simultaneously infect alliums and can include up to two potyviruses, *Onion yellow dwarf virus* (OYDV), *Shallot yellow strip virus* (SYSV), three carlaviruses, *Garlic common latent virus* (GCLV), *Garlic latent virus* (GLV) and *Shallot latent virus* (SLV) and six allexiviruses, *Garlic viruses A, B, C, D* and *X* (GVA, -B, -C, -D and –X) and *Shallot virus X* (ShVX). The potential presence of multiple virus infections complicates diagnostic testing. Furthermore, virus sequence diversity within each species can be quite significant. This makes the development of virus-specific RT-PCR diagnostics difficult as there is a risk that specific primer design may not encompass all genetic diversity, particularly, where the complete diversity is unknown.

A robust multi-target diagnostic procedure was developed in the project using generic RT-PCR assays followed by hybridisation with virus-specific probes in a dot blot assay. Unexpected thermodynamic interactions between heterologous targets for some probes resulted in the development of new stringency conditions for dot-blot assays. This work provides a useful multi-target diagnostic assay for allium viruses and expands the current knowledge on hybridisation based diagnostics.

Molecular screening of crop samples for virus was carried out using genus-specific RT-PCR-based assays followed by species-specific hybridisation assays using the method developed in this project. To do RT-PCR, the generic reverse primer Poty1 (GGATCCCGGGTTTTTTTTTTTTTTTTV; Gibbs and MacKenzie, 1997) was used to generate cDNA for all three virus groups. The cDNA was subsequently amplified in PCR using one of three different forward primers. For potyviruses this was U341 (CCGGAATTCATGRTITGGTGYATIGAIAAYGG; Langeveld et al., 1991), for allexiviruses it was pGV3 (TGGNCNTGCTACCACAANGG; Chen et al 2004) and for carlaviruses it was AlcarF (TGCTGCYTTTGATACYTTCGAT; this study). For cDNA synthesis, 2 µL of TNAE was incubated with 20 pmol of the Poty1 primer and 8 µL of water at 80 °C for 10 min then quenched on ice. The remaining reaction components of 5 × first strand buffer, 0.01 M DTT, 50 U of SuperScript III reverse transcriptase (Invitrogen), RNase inhibitor (10 U Invitrogen) and water to a final volume of 20 µL were added to each tube and reactions incubated at 50 °C for 45 min then 70 °C for 15 min. For the PCR assays, 2 µL of cDNA was incubated with 10 × PCR buffer, 400 µmol dNTPs, 1.75 mM $MgCl_2$, 1 U of Taq DNA polymerase (Invitrogen), 10 pmol of Poty1 primer and 10 pmol of generic forward primer (Alcar, pGVT or U341). Reactions were incubated at 94 °C for 1 min, then 35 cycles of 94 °C for 20 s, 56 °C for 60 s and 72 °C for 60 s, followed by a final incubation at 72 °C for 5 min.

PCR amplicons were subsequently analysed by dot blot assay using virus-specific DIG-labelled probes. To prepare dot blots, a 1 µL of denatured PCR product was applied to Hybond-N+ (GE Healthcare) membrane using a grid pattern. To denature the PCR products 5 µL of PCR product was added to 45 µL of denaturing solution (0.44 M NaOH, 0.011 M EDTA) and heated to 95 °C for 5 min. Membranes were washed in 2 X SSC for 5 mins and air-dried. The membranes were prehybridised by incubation with DIG-easy Hyb solution (20 mls/100 cm^2 Roche) in a glass hybridisation tube at 42 °C for 30 min with rotation. Excess pre hyb-solution was removed and replaced with (3.5 mL/100 cm^2) DIG-easy-Hyb with 1 µL/mL of denatured probe (heated to 95 °C for 5 min and added while hot to the DIG-easy Hyb) added, followed by overnight hybridisation at 42 °C with rotation. Following overnight incubation, membranes were washed using the appropriate stringency conditions.

To visualise the bound probe, membranes were developed using an anti-DIG alkaline phosphatase conjugate with a BCIP/NBT colour substrate (BioRad), following the DIG-detection kit (Roche) as per manufacturer's instructions. The colour substrate was left to develop for at least 30 min, after which the reaction was stopped by washing membranes in distilled water.

Fusarium (Twisted disease – Moler disease) in shallot

A focussed field study was conducted to determine the response towards Moler disease (Fusarium complex) of the common shallot cultivars grown in Nganjuk (East Java) and Bantul (Central Java) districts, in two different growing seasons. In Nganjuk and Bantul districts, field surveys of disease incidence were conducted in the wet and dry seasons. Field observations of disease incidence were initiated 2-3 weeks after planting at two (2) week intervals for six (6) weeks. Surveys were also done by observing Moler disease in shallot fields in the key production areas of Brebes (Central Java) and Cirebon (West Java). Surveys were carried out twice, during the wet and dry seasons. At each district five sub-districts were surveyed and and shallot crops were observed at about one month after planting. At each survey site the planting was divided into five blocks and in each block the percentage of Moler disease from 100 shallot plants was determined.

Moler disease symptomatic plant surveys were conducted in Batu and Nganjuk (East Java), Kulon Progo, Bantul, Gunung Kidul, Sleman (Yogyakarta), Temanggung,

Pemalang and Brebes (Central Java), Cirebon (West Java), and Enrekang (South Sulawesi) districts. Diagnostics, including molecular identification, were developed to identify the complex of pathogens that result in Moler disease in shallot.

Other Foliar diseases in shallot

A survey of foliar pathogens of shallot was conducted in Bantul and Nganjuk in the wet and dry seasons. The disease incidence was determined by calculating the percentage of infected plants. Leaf samples were collected from each site and taken to the lab where an *in situ* observation was done to check the spore morphology, followed by single spore isolation. Further research on fungal pathogen identification was conducted using Koch postulate and molecular procedures. Molecular identification was done only on the pathogenic isolates collected, post the Koch Postulate procedure, by PCR technique using the specific primers for *Alternaria* consist of forward primer: Dir1ITSAlt (5'-TGT CTT TTG CGT ACT TCT TGT TTC CT-3') and reverse primer: Inv1ITSAlt (5'-CGA CTT GTG CTG CGC TC-3'). Further to using specific primers for Alternaria, rep-PCR using ERIC2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') and BOXA1R (5'-CTA CGG CAA GGC GAC GCT GAC G-3') primers were conducted for grouping the isolates. Further analysis was conducted by sending the specific PCR products of Alternaria isolates for DNA sequencing.

Bacterial diseases of shallot in Indonesia were observed in the farmer fields and post-harvest in storage and in markets. Field sample collection was done by selecting plant clusters with wilting symptoms and for storage samples by selecting bulbs with soft rots from storage or markets in Bantul, Nganjuk, Cirebon and Brebes. Bacteria were then isolated from the samples using common medium of nutrient agar for soft rot bacteria or (LB) medium with 50 lg⁄ml cyclohexamine for *Pantoea ananatis*. Gram reaction, tobacco hypersensitivity tests, potato tuber and shallot bulb soft rot tests, and pathogenicity test on shallot plants were conducted. Further identification was conducted by rep-PCR technique using the primers of BoxA1 and ERIC. Each cluster was then subjected to amplification of 16SrDNA, mdh and rexA genes followed by sequencing of the PCR products. For detection and identification of *P. ananatis*, the primers of ITS1 and EC5 were used. The PCR products were subjected for DNA sequencing. The sequence data were then analysed using BLAST to get close homology with published sequences. Further specific primers were used to isolate *P. ananatis*.

5.3 Objective 2.2 Field sampling and assessment of soil fertility limitations in shallot/chilli production.

Partial nutrient budgeting in shallot

The partial nutrient budget in shallot was conducted as per a defined protocol developed by DAF. In short, the crop yield and nutrient removal were calculated and matched with the amount of applied fertiliser to assess the efficiency of crop fertiliser use. This activity was carried out by IVEGRI and UGM.

The following information was collected to assess the crop nitrogen uptake and fertiliser use efficiency:

- Crop yield (t ha^{-1}) including the yield of crop residues.
- Shallot foliage and bulb dry matter concentration determined and expressed as a percentage.
- Dried foliage and bulb samples analysed for N, P and K.

- Amount of nitrogen removed in the harvested product (kg ha^{-1}) determined.
- Amount of nitrogen returned to the soil system as crop residues (kg ha^{-1}) determined.
- Quantity of nutrients applied as fertiliser (kg ha^{-1}) determined.

Initially six collaborating farmers in Brebes and six collaborating farmers in Cirebon were selected to participate in the survey. A further six collaborating farmers were selected for both Nganjuk and Bantul. At each farmer site the field was divided into four small subplots and yield measured for each of the four sub plots. Samples were then taken from each and the average for each farm determined to assess nutrient removal and fertiliser use efficiency. The budgeting required the calculations of both the harvested shallot bulb yield as well as the yield of the crop residues (shallot foliage or tops).

At crop maturity shallot bulb and foliage yield, DM% and N content were determined. The fresh yield per ha for the bulbs and plant tops was adjusted by the furrow percentage area (24.7%) associated with the irrigation furrows of the Surjan system. The samples taken from the harvested plots were analysed for N, P and K in Indonesia and were also sent to Australia where a full nutrient analysis (N, P, K, Mg, Ca, S, Fe, Mn, Zn, B and Cu) was conducted as well as Na and C.

The nutrient uptake and removal per ha in the bulbs and crop residues was calculated by determining the dry matter yield (fresh yield multiplied by DM%) and multiplying by the nutrient concentration in the dried tissue sample. Fertiliser application records were obtained from each farmer, particularly including the amount and type of fertiliser that was applied and also manure or compost applications. The total kilograms of each nutrient applied (kg ha^{-1}) were calculated. The nutrient uptake (kg ha^{-1}) of the marketable product and field residues was obtained by multiplying the dry matter yield by the sample nutrient concentration (for N, P and K this is expressed as a percentage). A nutrient use efficiency was calculated as a percentage of nutrient removed divided by nutrient applied. There was no allowance for mineralisation of N and P from soil organic matter, nor for inorganic nutrient content of the soil.

Shallot Nitrogen rate trial - Cirebon

An experiment was conducted by IVEGRI in a farmer's field in Ciledug (12 m asl.), Cirebon, West Java to evaluate the effects of seven nitrogen rates (0, 40, 80, 120, 160, 240 and 320 kg N ha^{-1}), applied as urea on the growth of shallot cv. Bima. Field plots were prepared by making beds and furrows. The beds were 5.0 m x 1.5 m and the furrows were 0.50 m wide. The experimental plots consisted of two beds and each bed consisted of eight rows with 25 plants planted in each row. Therefore a total of 200 plants were planted in each bed and a total of 400 plants were planted in each experimental plot. Prior to planting, compost at a rate of 4 t ha^{-1} was applied in each bed by broadcasting it. Standard farmer agronomic practices for other nutrients and spraying were followed. Preplant soil samples were taken and soil and plant samples were also taken at maximum growth in order to determine the nutrient uptake, especially nitrogen.

Data gathered included plant height, number of shoots per plant, number of leaves per plant as well as fresh and dry weights of the foliage and bulbs at harvest. Sampling for fresh weight and dry matter for the shallot bulb and foliage was carried out at 37 and 56 DAP with a final harvest at 56 DAP and yield determined. Plant samples were dried and analysed for nitrogen at the IVEGRI laboratory.

A further nitrogen response experiment was conducted on the second cropping cycle for shallot using N rates from 0-400 kg ha^{-1} and a similar methodology to that described above. The experimental designs were randomized and complete blocks each replicated 4 times. Data was analysed using analysis of variance and curvilinear regression using SigmaPlot.

Garlic Nitrogen trials Queensland Australia

A series of field experiments were conducted at the Queensland DAF Gatton Research Facility to evaluate N responses in garlic (cv. Glenlarge) and in combination with plant density. The trial sites were initially planted to forage sorghum, which was bailed and removed from the site to minimise the soil residual nitrate levels and variability. The N rates imposed consisted of 0, 40, 80, 120, 160, 200 and 240 kg N ha^{-1} and in the experiment that looked at plant density, plant populations of 133, 200 and 266 thousand plants ha^{-1} were imposed. Reference leaf and soil samples were collected for each replicate of each treatment. At harvest, yield was determined and bulb and foliage samples dried and analysed for all elements. The experimental design was a split-plot with main plots as N treatments and sub-plots as plant density each replicated 4 times. Data was analysed using analysis of variance and curvilinear regression using SigmaPlot. Seed-bulbs were retained from each N treatment at 133 thousand plants per ha and planted in the following year all at an N rate of 180 kg N ha^{-1} to evaluate the impact of N treatment in the previous year on subsequent garlic crop yield. This experiment was a randomised complete block with 4 replicates.

Experimentation within the project showed that the ammonium fertiliser forms (including urea) negatively impacted on garlic growth. Experimentation was also conducted to evaluate the effects on garlic growth of different forms of N fertiliser, including urea, ammonium and nitrate forms. Nitrogen fertilisers were applied as prilled forms spread evenly over the plot by hand and irrigated with a lateral boom irrigator delivering about 15 mm at each application. A composite reference leaf sample was collected from each plot and analysed for total N and nitrate N. Trials were harvested and fresh yield DM% and dry matter yield determined. Bulb samples were analysed for nutrient concentration.

5.4 Objective 3: To develop a system to produce and supply clean pathogen tested shallot (Indonesia) and garlic (Australia and Indonesia) seed.

Collection of shallot and garlic cultivars in Indonesia and Australia.

A large suite of shallot and garlic germplasm was assembled in both countries and evaluated for agronomic performance and adaptation. Shallot varieties were collected from different shallot production areas in Yogyakarta (Bantul and Kulon Progo), East Java (Nganjuk and Probolinggo), Central Java (Brebes) and West Java (Cirebon). Shallot and garlic seed-bulbs were also imported from AVRDC to Indonesia and seed-bulb of 29 garlic varieties imported into Australia. In Indonesia, the shallot and garlic seed-bulb collections were planted in the screen-house or in the field of farmers land. In Australia, the garlic varieties were propagated under laboratory conditions to check disease status and subsequently under field conditions. A further three tropical shallot varieties (East West Indo cvs. Tuk Tuk, Sandren and Locananta) were imported into Australia for evaluation at both the DAF Gatton research facility and in a commercial field.

Selection of virus-free lines and virus elimination from alliums (shallot and garlic).

The use of virus-free bulbs as initial planting material reduces disease development and increases shallot crop yields. Viral diseases are easily transmitted via infected bulbs from one generation to the next and from one region to another. Cultivation of virus-free seed-bulbs is considered the most effective method of controlling viral diseases. *In vitro* culture, using shoot tip culture, is the most widely used method to obtain virus-free bulbs

(Al Maarri *et al*., 2012). Shoot-tip culture can be combined with several techniques, such as thermotherapy, chemotherapy, electrotherapy, and cryotherapy to improve efficiency of virus elimination. Among them, thermotherapy and chemotherapy are the most common methods used for virus elimination (Rout *et al.,* 2006). Efficient techniques need to be developed to get virus-free bulbs in order to improve the quality of shallot seed-bulb in Indonesia. Therefore, research has been done to develop a method of producing virus-free shallot bulbs using combinations of shoot tip culture, thermotherapy, and chemotherapy.

Shallot bulbs were washed and cleaned prior to shoot-tip culture. The outer layer and necrotic stem base of the mature bulb were removed. The bulbs were washed using detergent and rinsed with tap water. The bulbs was then soaked in 2 g L^{-1} fungicide and 2 g L^{-1} bactericide then incubated overnight. Subsequently, bulbs were soaked in 20% sodium hypochlorite and shaken at 150 rpm for 20 min. The bulbs were then triple rinsed in sterile distilled water in a laminar flow cabinet and the two outer layers were removed, and the bulb then soaked again in 10% sodium hypochlorite for 10 min and triple rinsed. The bulbs were excised to 5 mm and the cut surfaces dipped in 5% sodium hypochlorite for 5 min then planted temporarily in MS0 medium to prevent explant damage. The 5 mm explants were then further excised into 3 mm, 2 mm, and 1 mm using sterile needles and binocular microscope. Each explant size was cultured on basic MS medium and incubated for 4 days. Explants that were contaminant-free were transferred into medium to induce shoot development and used later for thermotherapy. Shoot-inducing medium was made by MS (Murashige-skoog) medium added with 2ip (2 ppm), GA3 (0.3 ppm), 0.3% sucrose, and gelrite 2 g L^{-1}. Root-inducing medium was made by MS medium with gelrite 2 g L^{-1} and 0.3% sucrose.

The heat treatment consisted of three temperature regimens of 30 ^{o}C and 37 ^{o}C constant temperature and a 25 ^{o}C as a control treatment. Two conditions of thermotherapy were examined including incubation in homogenous and heterogenous conditions. For the heterogenous condition, the explant was incubated at 25^{o}C for the first 2 week (s), then in 30^{o}C for the next week, then in 37^{o}C for the following week. For the homogenous condition, explants were incubated at each temperature (30 ^{o}C, 37 ^{o}C, and 25 ^{o}C) for 4 weeks with a photoperiod of 16 h light (52 µm s^{-1} m^{-2}). All plantlets that survived the thermotherapy treatment were tested for potyvirus and carlavirus using RT-PCR as previously described.

Explants for chemotherapy were obtained from the previous thermotherapy treatment, i.e. plantlets that remain infected by potyvirus and carlavirus. The shoot tips of 2 mm were excised from plantlets then planted in the medium containing ribavirin (Virozole, Sigma Aldrich,US) at different concentrations including 10 ppm, 25 ppm, and a control with no ribavirin. These explants were then incubated at temperatures of 30 ^{o}C for 4 weeks under a 16 h light photoperiod (52 µm s-1 m-2). After 4 weeks, plantlets were transferred into MS medium without ribavirin and incubated at 25 ^{o}C.

The first experiment was conducted using a factorial completely block design with two factors, i.e explant size (1 mm, 2 mm, and 3 mm) and temperatures (30 ^{o}C and 37 ^{o}C, control 25^{o}C), with 3 replications and 10 explants per replicate. The second experiment was conducted using completely randomized design of thermotherapy combined with several concentrations of ribavirin (0, 10 ppm and 25 ppm) and replicated three times. All data were subjected to analysis of variance and significant differences of the means were evaluated using Student's t-test or Duncan's multiple range test. Characteristics of plantlet growth were observed, including percentage plantlet survival, average number of leaves and average number of roots.

True seed propagation from native Indonesian shallot varieties.

This study was conducted by UGM in the priority area of Bantul and included flower initiation, floret fertility and seed fertility evaluations of three (3) native shallot cultivars (i.e. Biru, Crok and Tiron) and laboratory analysis of traits (chromosome identification, pollen fertility, viability and variability identification of shallot seeds). Fertility of three native shallot cultivars was tested in ovules by observing the seed set under open pollination. Pollen fertility of shallot was assessed using aceto-carmin staining, while chromosomal characteristics of shallot were observed using Feulgen staining. The viability of shallot seeds was evaluated as germination rates of seed. Variability in shallot seeds was identified by morfological characteristics and RAPD analysis.

Preparations for a pilot shallot and garlic seed production scheme and strategies to extend time for reinfection.

Two experiments were conducted to evaluate planting and sowing techniques to optimise the commercial use of TSS.

The first experiment evaluated the effect of plant growth regulator (PGR) and planting density against viral infection and the production of origin bulbs of true shallot seed in the highlands. A field research experiment was carried out from August 2017 to April 2018 at the Balitsa Lembang Experimental Facility (plateau 1250 asl) using the Trisula variety. The experimental design was a factorial randomized block design with two factors replicated three (3) times. The first factor type of PGR consisted of: A.1. BAP concentration of 50 ppm, A.2. NAA concentration of 50 ppm, A.3. GA3 concentration of 50 ppm, A.4. Control. The second factor was planting density and included seed rates of 5 grams m^{-2}, 7 grams m^{-2} and 9 grams m^{-2}. Yield, germination, plant growth and the incidence of key viruses was determined and the data analysed by analysis of variance.

The second experiment assessed which true shallot seed sowing methods is most technically and economically feasible. A field trial was conducted in Brebes District, Central Java Province from April to September 2017. The experiment was a factorial randomized block design with two factors and four replications. The first factor was sowing method (S) consisting of three levels including; evenly spread on sowing media at 10 gram m^{-2}; (S1) line-spread (spread in a 1 m shallow furrow at 10 cm spacing) (S2) and soil-media cake seedling (S3). The second factor was the sowing age (U), which consisted of two levels of 30 days growth (U1) and 45 days growth (U2). During transplanting, seedlings from evenly-spread and line-spread sowing methods were planted one (1) seedling per planting hole. Meanwhile, each soil block of soil-media cake sowing method had 2-3 seedlings per hole.

Socio-economic evaluation of shallot production and seed supply.

This study was conducted by IVEGRI staff and three postgraduate students from UGM in the priority areas of Cirebon, Brebes, Bantul and Nganjuk. A review of existing studies, reports, and government statistics was carried out early in the research process to take stock of current knowledge and available information. From this, data gaps were identified and the information used to identify the primary data collection process, particularly the choice of fieldwork locations and key respondents. Secondary data was further reviewed during the report writing stages to support and illustrate the analysis and findings.

A structured questionnaire was developed and used for interviewing shallot traders in Cirebon, Brebes, Bantul and Nganjuk. This method is particularly suited to a rapid appraisal of shallot marketing systems, allowing the researcher to explore a wide range of issues and collect quantitative as well as qualitative data. It also allows the trader interviews to be tailored to their specific knowledge and willingness to spend time discussing different questions. The research team interviewed three categories of

traders (local assembly trader, inter-regional assembly trader/wholesale trader and retail trader); these being considered to have the most important role in linking producers and consumers in the shallot supply chain.

An analysis of the shallot supply chain was conducted in Java's key shallot-producing areas of Cirebon (West Java), Brebes (Central Java), Bantul (Yogyakarta Special Region), and Nganjuk (East Java). The analytical framework adopted, the research methods employed, the areas visited during the fieldwork, and the structure and content of the report reflect the main study objective, which is to inform supply chain upgrading interventions with the potential to improve the marketing of shallot in Java.

Key reference models were used to develop the analytical framework that guided this supply chain study. A selective approach to data collection and the choice of tools and methods for data analysis (SWOT analysis) was followed, taking into consideration the resources and time allocated to each study. The study covered a wide range of issues deemed important for understanding the structure of shallot supply chains as well as their conduct and performance. Emphasis was given to the constraints faced by supply chain actors and the opportunities available to them.

Some of the key areas and issues covered were: (a) description of chain activities, (b) traders" specialization, (c) description of trade and product flow, (d) price formulation and marketing costs, (e) price risk, (f) trading volume and procurement sources, (g) buyer, seller and transactions, (h) market risk, (i) quality, (j) storage and storage risk, (k) liquidity, (l) competitiveness, and (m) constraints for development. An analysis of costs and margins was conducted.

5.5 Objective 4: Evaluate, develop and promote improved shallot and chilli integrated crop management strategies.

Resistance screening for PYLCV resistance in chilli

The initial PYLCV virus isolate was sourced from Brebes, Central Java. The virus isolate was transmitted and propagated on chilli pepper using a whitefly transmission method. The identity of the virus isolate was confirmed by a polymerase chain reaction (PCR) technique using a Geminivirus universal primer (SPG1/SPG2), followed by direct sequencing of the PCR product. Sequence data showed that the virus isolate had the highest homology (98%) with *Pepper yellow leaf curl Indonesia virus* (GenBank #AB267834.1).

Bemisia tabaci was collected from eggplant (*Solanum melongena*) in Bogor, West Java. Whiteflies were reared on healthy cotton plants (*Gossypium hirsutum*) grown in insect cages in screen-houses under 15 Watt lighting. Morphological identification showed that the pupal stage of reared whiteflies had vasiform orificae, which is the most important trait in the taxonomy and systematics family of Aleyrodidae. Molecular identification was done based on sequence analysis of the mtCO1 fragment from a single *B. tabaci* extraction. Sequence data showed that whiteflies have 100% homology with *B. tabaci* complex sp. Asia which was confirmed by GenBank (KJ778614.1).

Capsicum (*C. chinense*) seedlings were inoculated with PYLCV using *B. tabaci* at four (4) weeks after planting (4 leaf stage) following the method of Ganefianti *et al.* (2008). The whiteflies were given access to PYLCV-infected chili pepper plants for 24 hours. After the acquisition access period, the whiteflies were collected individually using an aspirator and transferred to healthy plants for 48 hours (inoculation access period). Ten adult whiteflies per plant were used for the inoculation access period. After the

inoculation access period, the whiteflies were removed, and the plants were sprayed with an insecticide and held for symptom development in the screen house. The Incubation period, disease incidence and severity were recorded. Disease incidence was recorded as the percentage of plants showing typical symptoms of PYLCV. Disease symptoms were scored based on a five (5) point scale, i.e. 0= no symptoms, 1= yellowing, 2= yellowing and leaf curling, 3= yellowing, leaf cupping with leaf curling, upward or downward, 4= yellowing, leaf cupping with leaf curling, upward and downward, and 5= yellowing, leaf curling, stunting (Ganefianti *et al.,* 2008). A large range of germplasm (>50 accessions) was screened to identify resistant genotypes.

Evaluate and develop integrated crop management strategies for shallot and chilli.

Effects of border crops, insecticides, and beneficial microbes on incidence of PYLCV in chillis

A field experiment was conducted in Pusmalang Village, Wukirsari, Cangkringan, Sleman Regency, Yogyakarta. The site was selected because it had been an epidemic spot for PYLCV in the previous years. Two treatment combinations were evaluated for their effectiveness in disease control and included border crops and insecticides or beneficial microbes. Border crops of corn and yard long bean were compared with conventionally planted (no border) chilli pepper. Application of the commercial insecticides and mycorrhiza was compared with mineral oil. The experimental design was a randomized block design with three replications.

A further field experiment evaluated a commercial PYLCV susceptible cultivar ('Pilar') and a potentially resistant line (IPB C12) in combination with border crops of corn, mineral oil application and commercial insecticide application. The experimental design was a split-plot design with three replications and borders as the main plots and cultivars as sub-plots.

Response of shallot cultivars to Moler disease after bulb treatment

This experiment evaluated the effectiveness of various bulb treatments against *Fusarium* infection and included hot water treatment, biofertilizer, and fungicide on four shallot cultivars. This research was done in the greenhouse using a Completely Random Design (CRD) with two factors. The factors included 4 shallot cultivars, i.e., Tiron, Crok, Trisula, and Kuning and eight (8) shallot bulb treatments with three (3) replications. The control treatments included submerging bulbs in aquadest as positive control for *Fusarium* sp. inoculation (P1) and the negative control was without *Fusarium* sp. inoculation (P2). The other treatments included submerging bulbs in hot water at 45^{o}C for 15 min (P3), 30 min at 45^{o}C for (P4), 50^{o}C for 15 min (P5) and at 50^{o}C for 30 min (P6), bulbs immersed in fungicide (P7) and a treatment of bulbs immersed in diluted biofertilizer (P8). Yield and disease incidence were assessed and data analysed using analysis of variance.

The effect of nitrogen fertilizer on nutrient uptake and shallot Moler disease

This research aimed to evaluate the effect of urea fertilizer on shallot growth, N uptake in inoculated and sterile soil on the incidence of Moler (Twisted leaf) disease in shallot. This research was conducted in a screenhouse at the Faculty of Agriculture, Universitas Gadjah Mada using a Randomized Complete Block Design (RCBD) with two (2) factors and four (4) replications. The first factor was two soil treatments, including a sterilised soil and a sterilised soil inoculated with *Fusarium solani*. The second factor was rate of N application and included N rates of 0 (N0), 50 (N1), 100 (N2), 150 (N3) and 200 (N4)

kg N ha^{-1} applied as urea. Yield and disease incidence were assessed and data analysed using analysis of variance.

Selection of shallot rhizobacteria for controlling Moler disease

Moler disease is one of the most important diseases on shallot. Various studies show that antagonistic rhizobacteria can be used as biological control of soilborne diseases. This study aimed to identify antagonistic rhizobacteria and their ability to suppress *F. acutatum* growth under *in vitro* and *in vivo* conditions at the Plant Disease Clinic and Glasshouse of Universitas Gadjah Mada, Yogyakarta. The study was conducted by isolating rhizobacteria from the rhizosphere of shallot's sourced from four (4) regions; Bantul district (Yogyakarta), Nganjuk (East Java), Cirebon (West Java) and Brebes (Central Java) district. Characterization of the rhizobacter was done based on physiological and biochemical traits, PCR based on 16S rRNA and Rep-PCR (BOX-PCR and ERIC-PCR). The *in vivo* test was carried out as two experiments using a completely randomised factorial experimental design. Treatment One included soaking shallot seeds in the rhizobacteria suspension for one hour before planting and treatment Two included watering plants with rhizobacteria suspension once every two weeks. The incubation period, disease incidence, plant height, fresh and dry weight of plants and total *Fusarium* population were measured. A greenhouse and field experiment were also conducted to identify the response of shallot cultivars to disease and biofertilizer application. Before field planting, seed-bulbs of 16 shallot cultivars were tested for the incidence of seed-borne diseases in the greenhouse with 20 plant replicates for each cultivar.

Objective 4.4 Evaluate shallot and chilli crop nutrient use efficiency and provide a position statement on current practices and recommendations on research needs in relation to fertiliser use.

Methodology for identifying chili germplasm with improved root systems and nutrient acquisition.

A series of field experiments were conducted over the course of the project to evaluate beneficial growth effects of grafting the standard Australian commercial capsicum (*Capsicum annuum*) cultivar Warlock over rootstocks of wild chilli (*Capsicum chinense*) accessions. The experiments assessed the yield potential and fruit quality of the grafted lines and compared this with the standard variety. The field experiments were conducted at the Gatton Research Facility of DAF.

Overall a total of 46 accessions were evaluated with warlock grafted over warlock (W/W) as the control treatment. The grafted seedlings were prepared by a commercial seedling producer and planted on beds (centres at 1.5 m), with plastic mulch and drip irrigation. Each plot was grown to maturity and harvested as a single full plant harvest of multiple plants. The plants were partitioned into foliage (stem and leaf) and fruit. Fresh weights of each of these components were determined and subsamples were taken, then weighed and dehydrated to determine dry matter concentration. Yield was calculated on the basis of a plant population of 44,500 plants per ha. Data was analysed using ANOVA. This method was essentially used in each experiment.

6 Achievements against activities and outputs/milestones

Objective 1: To characterise the agronomic practices of shallot-chilli-rice production and supply systems in key areas across Java.

No.	Activity	Outputs/ Milestones	Completion date	Comments
1.1	Review of available local information and scientific literature and reports. (PC and A)	Literature review completed	May 2013	A comprehensive literature review of shallot production and research in Indonesia was completed (appendix 1).
1.2	Conduct strategic surveys of shallot chilli agronomic, production and marketing practices. (PC)	Project management team evaluation of literature review to identify gaps and inconsistencies.	July 2013	The literature review was circulated for comment with no major changes required.
		Complete base-line (benchmarking) survey of farmers agronomic practices.	Oct 2013	The baseline survey was conducted with approximately 50 farmers in Cirebon, Brebes, Bantul/Yogyakarta and Nganjuk.
		Survey to evaluate the shallot chilli value/market chain analysis and existing seed supply systems.	Oct 2013	The survey of farmers and seed supply chain has been completed and is included in the appendices.
		Information reported and reviewed by project team.	Dec 2013	Completed.
1.3	Conduct stakeholder awareness workshops. (PC)	Awareness workshops held for grower and advisory/extension people.	Feb 2016	A series of meetings have been held in each of the key areas (Cirebon, Brebes, Bantul/Yogyakarta and Nganjuk) throughout the project.
		Project first annual review meeting held.	Jun 2013	Annual review meetings were held each year of the project and the Mid-project review was completed in October 2014 and a final review meeting held in October 2016 and a project showcasing held in February 2019.

Objective 2: To identify and quantify the incidence of significant pathogens and agronomic issues in allium and solanaceous crops in Indonesia and Australia.

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	Assessment of the incidence and distribution of pathogens. (PC and A)	Joint methodology development and training with Indonesian scientists.	April 2013	The survey protocol was completed Dec 2012 and implemented in the 2013 season. Initial virus methodology developed and training provided by April 2014.
		Completed Pathogen survey of shallot and chilli crops in target highland and lowland regions of Central and East Java	Oct 2015	This activity was completed and reported in the methodology and key results discussion sections.
		Australian surveys to accurately identify viruses and assess virus incidence in allium crops.	Oct 2015	This activity was completed and reported in the methodology and key results discussion sections.

	Assessment of the incidence and distribution of pathogens. (PC and A)	Conduct surveys in East Indonesia to assess the geographical distribution of PYLCV. Characterise other key begomoviruses.	June 2018	Surveys for major viruses infecting chilli pepper were completed in West Sumatra (Bukittinggi), West Nusa Tenggara (Lombok and Bima), South Sulawesi (Enrekang), Bali, Papua and West Papua,. Typical symptoms of begomovirus were observed in chilis in Lombok, Bima, Nort Maluku Timor and Sumba, and low incidence observed in Merauke (Papua) or Jayapura (West Papua). Begomovirus has been detected on all islands as the major virus, with *Pepper yellow leaf curl virus* (PYLCV) is considered as the predominant species. Other viruses detected from the field samples involves *Chili vein mottle virus* (ChiVMV), and *Cucumber mosaic virus* (CMV). All areas were surveyed for incidence of Moler disease (Fusarium complex) including Sumatra, Kalimantan, Papua and West Papua, WNT, Sulawesi, Lombok, Bali, Kupang (Timor) and Sumba. Moler disease was found in all areas including Palu and Merauke. In Merauke the farmers were originally from Java from where seed was brought and likely to be the source of infection.
2.2	Field sampling and assessment of soil fertility limitations in shallot/chilli production. (PC and A)	Protocol for collecting baseline plant and soil test data jointly developed.	Oct 2013	The protocols for collecting samples were developed jointly by the project team. A nutrient budgeting protocol was developed specifically for the Indonesian Shallot chilli (Appendix 3). Training was conducted in use of the nutrient budgeting protocol.
		Completed collection and analysis of plant and soil samples.	Oct 2014	The nutrient budgeting exercise that included the collection of soil and plant samples was completed.
		Assessment of potential fertility limitations in Brebes, Cirebon, Yogyakarta and Nganjuk.	Oct 2015	A report on this activity has been completed that includes the potential for nutrient limitations in shallot chilli production systems. It also presents a survey on the nutrient budgeting in shallot cropping that highlights excessive nitrogen application is a major issue.

PC = partner country, A = Australia

Objective 3 To develop a system to supply clean pathogen tested shallot (Indonesia) and garlic (Australia) seed and evaluate cultivar performance.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Collection of shallot and garlic cultivars in Indonesia and Australia. (PC and A)	Collection of shallot germplasm in Indonesia	June 2016	The collection of shallot germplasm was an ongoing activity for the project partners over the duration of the project. A suite of about 20 shallot varieties was imported from AVRDC under the project. IVEGRI maintains a suite of about 80 shallot cultivars at the Lembang research station in Java.
		Collection of short day garlic germplasm assembled from Indonesia and Australian growers.	Sept 2015	Similarly there has been ongoing collection of garlic germplasm and evaluation of this material. About 28 short day (tropically adapted) garlic varieties were imported into Indonesia from AVRDC but did not show strong viability due to seed age (old seed) under the tropical conditions. In Australia, the same suite of garlic varieties were imported and in the first instance these did not survive the post quarantine treatment (2013-14). A further importation was completed with a different post-quarantine protocol (2016) and this material has been regenerated under laboratory conditions and checked for potential new viruses and subsequently field generated (2017). A replicated experiment of this material has been completed. Non-replicated and replicated trials have been conducted to identify optimal adaptation windows for planting this material.
		Incidence of allium viruses and other pathogens assessed in collected germplasm.	Sept 2016	The shallot germplasm of IVEGRI has been profiled for virus incidence. The garlic germplasm in Australia has similarly been checked and showed high incidence of all virus groups. The garlic germplasm imported from AVRDC to Australia has been re-propagated and has been checked for virus infection prior to infield propagation.
		Trials completed to evaluate IVEGRI shallot germplasm in farmer fields in Java and possibly Sulawesi and Lombok.	June 2018	This activity could not be completed because of the limited availability of germplasm material in IVEGRI.
		Complete trials in Australia to evaluate garlic germplasm sourced from AVRDC	June 2018	Field trials of the material (28 varieties from AVRDC) have been completed in 2017 and 2018 over a range of planting windows. As expected there is a wide range of adaptation across the germplasm. A PhD student is now researching the adaptation of the material to earlier plantings in Australia.
		Send shortday garlic varieties from Australia to Indonesia for assessment under Indonesian conditions	June 2018	This has not been achieved yet but negotiations are under way to forward the germplasm to Indonesia. The process requires a considerable timeframe due to Indonesian importation protocols.
3.2	Selection of virus-free lines and virus elimination from alliums (shallot and garlic). (PC and A)	Conduct training course/workshop in tissue culture and virus elimination in Indonesia at IVEGRI.	Sharon can you add detail	This activity is completed and reported in the methodology and key results and discussion sections.
		Confirmation of virus status in allium germplasm.	April 2013 but ongoing	This activity has been completed all germplasm has some level of virus infection ranging from low infection (about 5%) to high infection (>98%) depending on the virus and crop (shallot or garlic).
		Virus elimination in allium germplasm and maintenance of lines in secure tissue culture conditions.	Sharon can you add detail	This activity is completed and reported in the methodology and key results discussion sections

		Trials conducted in Australia and Indonesia to compare effects of virus infection in planting material on crop productivity.	Nov 2016	The tissue culturing of the alliums has not generated enough material to complete these trials. Trials to evaluate this have been completed in Australia that compare 4 generations of shallot starting with true seed (virus free) and also in Indonesia. The trial in Indonesia was decimated by disease and spodoptera.
		Project team meeting to review trials.	Feb 2016	Completed at various times.
3.2	Selection of virus-free lines and virus elimination from alliums (shallot and garlic). (PC and A)	Complete trials to evaluate the impact of viruses on shallot/garlic crop productivity. (Brebes and Bantul and Imogiri)	June 2018	In Australia, the tissue culturing of garlic germplasm has not successfully removed viruses yet. Other research is developing real time PCR for quantifying virus titre to identify the impact of virus load in reducing yield. In reselecting healthy garlic lines 3 viruses have been removed from Australia's main subtropical variety and yield of this line is about 17 t ha^{-1} compared with about 10 t ha^{-1} in the line with the full virus complement. Field experiments in Indonesia using TSS have shown that early generation material has no virus and subsequent infection increases virus incidence with reduced yield. Hot water treatment of shallot bulbs before planting reduced virus infection and improved plant yield. Tissue culture experiments of shallot and garlic has been partially successful in producing virus free plantlet/bulbs using thermotherapy in combination with chemotherapy or electrotherapy. In 2 student postgraduate studies at UGM explants of meristem culture sucessfully eliminated virus in the in vitro cultivation. This was confirmed in a further study and storing seed-bulbs for 4 weeks at 37^{o}C followed by 45^{o}C for 60 min in a waterbath prior to in vitro cultivation was successful in producing virus free planlets.
3.3	Preparations for a pilot shallot and garlic seed production scheme and strategies to extend time for reinfection. (PC and A)	Process developed and reviewed for reselecting seed-bulb. Protocol implemented with key farmers in each region.	Nov 2015 Not required	A shallot cropping protocol is published by the BPTP in Bahasa and this document has been translated into English. The activity was not required as it is a dedicated program under the BPTP.
		Geographical survey of virus incidence in allium crops to identify low pathogen regions.	Completed	This activity is completed and reported in the methodology and key results discussion sections. Further surveys (outside Java) have been completed under the project extension.

		Completion of a pathogen tested shallot seed-bulb plan including strategies to increase time for virus reinfection.	Dec 2016	In the context of the priority for the Indonesian Government the focus is on TSS production. Because of this change in direction and the commercialisation of TSS cultivars the reliance on pathogen testing (for vegetative seed) is not necessary. The ability to now produce TSS of Indonesian varieties provides a better method for producing pathogen free seed. The question now is how can TSS either directly or as early generation bulbs be included into a commercial production system to supply seed. This activity and concept is now being discussed and progressed in conjunction with East West Seeds. In Australia, DAF (Queensland) are now growing a better quality garlic seed line that supplies seed to the commercial industry.
3.4	Socio-economic evaluation of shallot production and seed supply. (PC)	Completed base-line survey of the existing shallot production and seed supply systems.	August 2013	The baseline shallot production survey and seed supply systems has been completed.
		Stakeholder participatory workshop on shallot seed needs assessment held.	May 2013	Stakeholder participation workshops have been completed in Brebes, Cirebon, Nganjuk and Bantul. Presentations on the findings were made in each region over the course of the project. A comprehensive survey of farmer perceptions of seed supply and quality was completed. Essentially this confirmed that poor seed quality is a serious issue for shallot farmers in all the key shallot production areas of Java.
		Completed on-farm participatory demonstration trials and training using the FFS.	Dec 2016	This activity was renegotiated at the mid-term review where it was realised that a large amount of background information was required to develop the appropriate FFS program. The FFS program is a dedicated training module of many formal classes and was not a realistic outcome in the context of this project. A meeting was conducted with farmers in the Bantul area and this has developed and trialled a draft FFS program that was supported by the input from the BPTP. The pilot program was delivered in Bantul in the 2015 season and subsequently refined and delivered in the Cirebon and Nganjuk.
		Qualitative assessment of users' perceptions of technology adoption characteristics.	Not delivered	Much of the research in the project has been basic research to characterise the problems including diagnostics and evaluations of current practices and validation of potential interventions (eg. nitrogen and fusarium management). Though planned at the outset of the project the goal of achieving this was not realistic.
		Completed assessment of the techno-socio-economic viability of the proposed interventions.	Not delivered	Refer to above comments.
3.5	True seed propagation from native Indonesian shallot varieties. (PC)	Cataloguing of existing information on IVEGRI's Indonesian TSS varieties.	Oct 2015	This activity is completed and reported in the methodology and key results discussion sections of the final report.

	True seed propagation from native Indonesian shallot varieties. (PC)	True seed of Indonesian Shallot lines sent to Australia to further propagate large quantities of true seed to conduct trials in Indonesia. Dr Trimartini Patria and Retno to potentially travel to Australia to evaluate allium seed production and breeding. Australian expert to potentially travel to Indonesia to discuss allium breeding strategies and seed production.	June 2018	Restrictions on the access of the germplasm has not allowed importation of the indigenous Indonesian shallot varieties to Australia. However, quantities of commercial TSS cvs TukTuk, Sanren and Lokananta have been sourced from East West Seeds and trialled in Australia. The trials included replicated evaluations of the TSS over time as well as a commercial trial for which product was marketed in Sydney and Singapore. In Australia the product was very well received with good market acceptance and a high price $AUD 6.00 per kg. Dr Trimartini Patria took a new position in Jakarta unrelated to this project and Ms. Retno undertook postgraduate studies in Japan and were unable to make this trip.
		Research trials conducted by UGM and IVEGRI to identify improved agronomy and genetics for Indonesian TSS varieties and evaluate productivity in highland and lowland production regions..	June 2018	Research trials on this have been completed and were reported at the project showcasing February 2019. The Effect of ZPT and Planting Density on the production of origin bulbs of True Shallot Seed was evaluated at the Balitsa Lembang Experimental Facility (plateau 1250 asl) using Trisula variety from August 2017 to April 2018. An experiment was conducted on improved sowing techniques and transplanting for TSS cultivation. This evaluated and identified which true shallot seed sowing method is most technically and economically feasible. TSS production in the lowland area in Bantul was conducted during the second planting season (May/June-July/August 2018) when lower temperatures are recorded and resulted in limited flower formation and produced some seeds. However, the seeds had very low viability (<20%). In the upland areas where farmers grow shallot during the rainy season floral initiation was good but the prevailing wet conditions did not support polination and subsequent seed production. TSS production in closed plastic houses with insect pollinaters appears to be the best option but nonetheless results in severe infection by Stemphillium sp. and potential spore contamination in the seed. In Australia, productivity of the East West shallot cvs TukTuk, Sanren and Lokanata has been evaluated using TSS and seed bulb. Propagation using TSS results in considerably lower incidence of seed stems (an undesirable market trait) compared with seed-bulbs.
		Farmer TSS productivity trials completed in 4 key regions and FSS training and stakeholder workshops completed.	06/2015	This activity was renegotiated at the mid-term review to be deleted.

Objective 4 Evaluate, develop and promote improved chilli and shallot agronomy and disease management strategies.

No.	Activity	Outputs/ Milestones	Completion date	Comments
4.1	Screening germplasm for virus resistance. (PC and A)	Completed screening of pathogen resistant germplasm identified in ACIAR AVRDC project HORT/2004/048	Completed but ongoing	This material has been evaluated along with other new material. A chilli breeding and virology workshop was held at IPB Bogor for all partners (IPB, IVEGRI, UGM, DAF, UQ and WorldVeg) to discuss the current status in breeding for resistance in chili to PYLCV. The workshop has developed a pathway to further progress the activity.
		Generation of mapping populations and development of understanding of genetics of resistance.	12/2013	A cross between inbred parent lines and the resistant lines has been made. However the resistance in the best (nominally) resistant line ICB C12 was not entire meaning little can be done to advance the understanding of genetics until this is resolved. The ICB C12 is believed to have 3 genes to confer resistance to PYLCV so the identification of a 100% resistant line has not been achieved. This is essential before a mapping population can be developed.
		Integration of WTG-resistant chilli germplasm into early breeding populations.	04/2015	Not achieved in this project refer to the above comments.
		Screening of collected Indonesian and AVRDC shallot germplasm for tolerance to pathogens.	07/2014	Completed but results lack consistency to allow a reliable assessment of the genetics of resistance and the development of 100% resistant lines.
4.2	Evaluate and develop integrated crop management strategies for shallot and chilli. (PC)	Completed replicated research station trials to evaluate barrier crops effects on virus incidence.	Oct 2015	Trials evaluating the effects of barrier crops have been completed by IPB at Yogyakarta and Lembang; refer methodology and key results and discussion. The first experiment was evaluated during the mid-term review.
		Replicated trials evaluating effects of various treatments (e,g oil sprays, SARs etc) on whitefly populations and WTG levels.	Oct 2015	Trials evaluating the effects of barrier crops have been completed by IPB at Yogyakarta and Lembang; refer methodology and key results and discussion. The first experiment was evaluated during the mid-term review.
	Evaluate and develop integrated crop management strategies for shallot and chilli. (PC)	Conduct experimentation to evaluate strategies to better manage crop fertiliser inputs	June 2018	Further experimentation in Brebes on nitrogen rates has been completed but the results were not definitive. ▪ The absence of significant differences of the nitrogen application rates was probably due to the excessive use of fertilizer in the previous season. The present experiment was carried out after the first shallot planting season where excessive fertilizer application was made. ▪ In order to have the right conclusion especially for the N use efficiency in shallot grown in Alluvial soil a similar experiment should be carried out in the first planting season after paddy rice and not carried out in the second planting season. This data was presented at the project showcasing February 2019.
		Conduct pot and field experiments to evaluate strategies to better manage fusarium.	June 2018	Tasks have been completed and were presented at the showcasing and in the final report.

4.3	Evaluate productivity improvements associated with grafted chilli and containerized seedlings. (PC)	AVRDC training for Indonesian scientists on grafting methods in chilli.	04/2013	This activity was deleted early in the project based on partner discussions and the lack of drivers that would make it necessary (notably soil born wilt pathogens were not a major problem). The use of containerized seedlings is already practiced extensively.
4.4	Field surveying and benchmarking of nitrogen use efficiency. (PC)	Partial nutrient budget field surveys conducted in Brebes, Cirebon, Yogyakarta and Nganjuk .	Oct 2015	This activity has been completed and is reported. The data highlights excessive application of N, P and K fertilisers is a general problem in all production areas. Further research has demonstrated that shallot yield responses occur at N rates up to about 300 kg N ha^{-1} but fertiliser use efficiency decreases considerably with increasing N rate.
		Nutrient management workshops held in key shallot/chilli production regions in Java.	11/2014	Data has been presented to farmer forums in Bantul district.
	Genetic improvements for nutrient acquisition. (A)	Replicated field trials completed in Australia to identify root stocks that infer higher productivity and improved nutrient acquisition.	Oct 2016	A series of experiments on genetic improvements in pepper root systems has been completed and is reported. This research has identified 5 accessions of *Capsicum chinense* that confer greater productivity.
		Key project staff from Indonesia to visit Australia to evaluate and inspect trials and project meeting.	Aug 2014	A total of 10 Indonesian project partners attended activities associated with the IHC2014. The activities included the IHC2014 Virology Workshop, Vegetable farming systems tour and specialised laboratory training.
4.5	Project extension activities for priority issues in Shallot and Chilli	Project dissemination workshops held in Java, Sumatra and WNT	June 2018	This has also been completed and were presented at the project showcasing. The surveys, samplings and experimental field trials have been conducted with the direct collaboration and involvement of farmers or farmer groups. This interaction always involves extension and technology transfer for IPM to improve vegetable production. For the surveys and disease sampling the extension were single events with feedback to collaborating farmers, while for the field trials the extension activities were repeated every planting season over the period of cultivation. Details of this activity on ICM TOT (Training of Trainers) and/or workshop on shallot and chili are presented in Section 8.4 communication and dissemination.
				Field days were held in Australia in Garlic (2014, 2015, 2016, and 2017) and shallots (2017). Individual famer visits were hosted for garlic and shallot experiments in 2018. The project has sourced commercial quantities of TSS cultivars Sanren and Lokananta from Indonesia and assisted a large onion grower in the Lockyer Valley to grow product which he supplied to Sydney markets at a premium price and a further sample was sent to Singapore. Dr Stephen Harper presented research findings on improving garlic productivity at the Australian Garlic Growers Association annual meeting Albury Jul 2018. Prof. John Thomas presented research findings on virus impacts on garlic productivity at the Australian Garlic Growers Association annual meeting Albury Jul 2018.
		Farmer participatory workshops conducted in Java to identify motivations for chemical application, product knowledge (in relation to targeted pest) and information sources for chemical application.		Completed and reported.

7 Key results and discussion

7.1 Literature review and characterisation of the agronomic practices of shallot-chilli- production across Java.

Literature review summary

The literature review identified that shallot and chilli are the most important vegetables in Indonesia. Current yield of shallot crops in Indonesia is low at only 9.6 t ha^{-1} and well below the maximum achievable yield of over 20 t ha^{-1}. Though yield has increased over the last 20 years, there has been a stagnation or slight decline in yield in the last 10 years despite increasing levels of inputs. Alliums as a crop group represent about 90% of Indonesia's vegetable imports and this is mostly as shallot and garlic, both of which are substantial commodities in Indonesian diets. Consumer preferences for shallot quality include a large bulb with purplish to dark red skin colour, dry skin texture, strong pungency and a round shape. Yields of garlic are also very low at only 6.7 t ha^{-1}. Domestic production of garlic represents just 4% of the total local garlic consumption. About 415,000 tonne of garlic is imported annually and the value is equivalent to about 50% of Indonesia's domestic shallot production; hence there is a strong demand for garlic and a need to improve garlic productivity. Increasing garlic production is a major priority for the Indonesian Government.

The key constraints to shallot productivity include.

- Heavy reliance on vegetative propagation
- Poor quality seed-bulbs
- Unavailability of acceptable and affordable true-seed shallot varieties
- Poor seasonal conditions combined with susceptibility to many pests and diseases
- Low levels of shallot breeding activities to improve the genetic base for production

Seed-bulb quality and purity (trueness to type) for shallot planting material needs to be improved to increase yield. Most farmers keep their own bulbs for replanting, which even with good selection, is likely to result in high virus incidence.

The review identified that the majority of farmers apply excessive fertiliser, particularly N, where 85% of farmers apply more than 160 kg N ha^{-1} at an average yield of only 7.9 t ha^{-1}. At a dry matter concentration of about 15% and a tissue N content in the order of 2.5-3%, the bulb N uptake is only around 30-40 kg N ha^{-1}.

The key pests identified are *Spodoptera* and *Thrips tabaci*. With the recent identification of Iris yellow spot virus (IYSV) in Bogor (2013) the management of thrips needs careful consideration in the future and monitoring for incidence of IYSV is likely to be important. A large spectrum of diseases affect shallot in Indonesia including Anthracnose (*Colletotrichum gloesporioides)*, *Fusarium oxysporum* sp. Cepae), Purple Blotch (*Alternaria porii)* and Bacterial Rot (*Erwinia carotovora*). Also a range of viruses have been detected including *Onion yellow dwarf virus* (OYDV), *Shallot latent* virus (SLV), *Leek yellow stripe* virus (LYSV), and *Shallot yellow stripe virus* (SYSV) but the impacts on crop yield and their distribution is not known. The review identified that in controlling pests and diseases, shallot farmers apply mixtures of multiple pesticides at two to three day intervals, and at high concentrations. The field exposure to poor farm labourers, a high proportion of whom are women, represents a serious health and safety risk.

Surveys of shallot chilli agronomic and production and marketing practices.

A baseline agronomic and production survey was conducted in the four key production areas of Java (refer to methodology section 5), including the coastal districts of Brebes and Cirebon and the Eastern districts of Bantul and Nganjuk. The major source of learning and information for farmers was in the form of family (relatives), with about 70% of respondents indicating passed down knowledge was a very important learning preference in relation to their farming. Learning from other farmers was also important (about 40% of respondents) but extension workers were not a major source of information (only 7% of respondents used this outlet).

Regional differences were observed in the size of farming operations and tenure. The farm size in Cirebon was greater than that of the three other regions. In Cirebon, about 70% of farmers had more than 5000 m^2 cropped to shallot/chili but the proportion with this land area were much lower in Brebes, Bantul and Nganjuk (20%, 40% and 3% of respondents respectively). No farmers in Brebes had an area less than 2,500 m^2 but in the other regions the percentage respondents with less than 2,500 m^2 was about 30-60%. The land tenure in Cirebon was also different from that in the other three regions with a high proportion of farmers (87%) relying on land rental to support their farm business compared with 60-70% in the other districts. Consequently, there is very little incentive for them to invest in soil improvements, (eg. applying organic matter, composts, manures etc.) since the benefits from such an investment are perceived to be long-term in nature.

The farmers grow a wide range of vegetable and other crops including shallot, chilli, eggplant, sweet corn, cucumber, yard long bean, squash, bitter gourd, tomato, mustard, rice, peanut, maize, kangkong, chinese cabbage and spinach. However, shallot is the dominant crop and up to three crops can be grown on the one land parcel per year. In Java, shallot is grown mostly after the wet-season rice cropping cycle. However, in Cirebon and Brebes shallot is also frequently grown after two-years of sugarcane. At least once per year shallot is inter-cropped with hot pepper, usually immediately after rice or sugarcane. In the last 2-3 years, more farmers in Cirebon have rotated shallot with sweet corn. The cropping pattern in Bantul commences with rice in November (rainy season), followed by shallot mono-cropping (February-May), hot pepper mono-cropping (May-August) and a final shallot and hot pepper inter-cropping (August-November). In almost all locations, shallot is cultivated using the Surjan system which allows for better drainage and reduces the risk of flooding and water logging.

Shallot price fluctuations are considerable, and are mostly due to climatic conditions affecting supply and quality, inadequate storage facilities and speculative trading practices.

An informal seed-bulb supply system exists where farmers obtain most of their seed-bulbs through informal channels that include farmer saved seed, seed exchange among farmers and a local shallot seed-bulb market. This system accounts for about 95-100% of shallot seed-bulb supply and a critical weakness of this system is the potential for poor seed quality. Fertiliser application rates are excessive and the mean application rate across respondents was about 210 kg N ha^{-1}, 60 kg P ha^{-1} and 150 kg K ha^{-1}.

Pesticide use in shallot chilli agronomic and production

The issue of pesticide use was a serious concern identified in the survey and key data are presented and discussed here. All farmers surveyed used chemical control as their principal means of controlling pests and diseases (Table 7.1). However, mechanical control of pests or removal of infected plant material was also an important strategy in all four study sites and is usually carried out by women. Apart from mechanical control, other soft options including biologicals, botanical pesticides and physical traps are not used to a great extent.

In all four districts the direct interaction with other farmers is the most important source of farmers' knowledge of pest and disease control (Table 7.1). The role of extension workers, pest observers, input resellers (pesticide shops) and pesticide company field staff as pest-disease control knowledge sources was comparable. The significance of extension workers and pest observers varied from 26.7% to 50% across areas perhaps reflecting the quality of service in each area. In all areas, both pesticide sales people and company representatives were important sources of information on chemical control. The majority of farmers in all four study sites carry out routine field observations for pest incidence.

Table 7.1 Methods for pest and disease control and pesticide application data for shallot crops in a survey of shallot farmer fertiliser practices in the Cirebon, Brebes, Bantul and Nganjuk regions of Java Indonesia in 2012.

	Cirebon (% Resp) (n=30)	Brebes (% Resp) (n=30)	Bantul (% Resp) (n=30)	Nganjuk (% Resp) (n=30)
Pest/disease control method				
Mechanical (collecting pests, picking up infected leaves)	96.7	100.0	83.3	80.0
Physical (trap crops, light trap, pheromone)	23.3	3.3	3.3	0.0
Biological (parasitoid, predator)	0.0	0.0	0.0	0.0
Botanical pesticides	13.3	0.0	6.7	0.0
Chemical synthetic pesticides	100.0	100.0	100.0	100.0
Sources of knowledge for control method used.				
Own experience	23.3	16.7	26.7	6.7
Other farmers	56.7	63.3	43.3	53.3
Extension worker or pest observer	36.7	26.7	43.3	50.0
Inputs – pesticide shops	33.3	30.0	30.0	33.3
Pesticide company's field staff	36.7	43.3	23.3	20.0
Spraying based on calendar system				
Yes	33.3	16.7	26.7	30.0
No	66.7	83.3	73.3	70.0
Spraying interval				
2 days	0.0	20.0	30.0	10.0
3 days	93.3	80.0	36.7	23.3
4 days	3.3	0.0	0.0	6.7
5 days	3.3	0.0	13.3	13.3
6 days	0.0	0.0	20.0	3.3
>6 days	0.0	0.0	0.0	43.3
Average (days)	3.1	2.8	3.5	5.9

Approximately one-third of farmers in each location apply pesticides on a regular basis using a calendar based schedule. However, in Brebes and Cirebon, although the majority of respondents did not identify as using a calendar spray system, more than 90% of farmers sprayed on a 2-3 day cycle (Table 7.1) indicating a fixed spray routine. In

contrast to Brebes and Cirebon, farmers in Bantul and Nganjuk tended to use wider spray intervals with about a third of farmers in Bantul using a spray interval of five (5) days or greater and in Nganjuk 60% of farmers use a spray interval of five (5) days or greater. The variability in this application needs to be further understood to assess whether this is indicative of a higher level farmer knowledge, lower pest pressure or a function of prevailing environmental conditions that preclude spraying at tighter intervals.

Farmers were surveyed on a range of issues associated with pesticide product knowledge and safety in application. In general, the majority of farmers would read a chemical label prior to application but the nature of the information they are seeking was not defined (Table 7.2). That is, it is unknown whether farmers were simply reading the rate they needed to apply or were developing a more informed understanding of the product. In Brebes, Bantul and Nganjuk, 30-40% of respondents never checked the active ingredients on the label which would indicate that their reading of labels is largely superficial, probably simply checking the rate of application. Notwithstanding, in each of these three districts about 40% of respondents always checked the active ingredient. In the Cirebon district, the survey data suggested a higher level of care in understanding products where greater than 70% of respondents always read the label and checked the active ingredient. Adherence to the label recommended rate was not strong with 20-30% of farmers never using the recommended rate and about 50% only sometimes adhering to the recommended rate. Data was not collected, but presumably this implied farmers used rates in excess of that recommended on the label but further surveys would be required to tease this out. More than 90% of farmers would always mix more than one chemical.

About 70-90% farmers (especially in Cirebon and Brebes) do not adopt full precautionary measures for personal protection from chemicals, including full body protective coverage, masks, gloves and caps, when using all chemicals. Mostly the farmers perceive that pesticide poisoning symptoms are normal and they get used to them. Farmers in Bantul and Nganjuk appeared to be more aware of pesticide health risks as indicated by a relatively high percentage of farmers who wear masks when spraying pesticides. For most farmers protective equipment is simply a mask.

Overall, about 10-30% of farmers in each region indicated they never read pesticide labels before spraying, never checked the active ingredients on the label, never targeted pesticide use based on specific pests or diseases and never applied the recommended rate. This is alarming considering that toxicity of pesticides to human health and the environment and exposure to pesticides is one of the most important occupational risks among farmers in developing countries. The average spray interval of only three days in Brebes and Cirebon is a concern since these areas are the major production centres for shallot and chilli and are on the coastal zones where environmental impacts are likely.

The use of pesticide mixtures (cocktails) is quite common for most farmers in the four study regions. An evaluation of the pesticide mixtures applied by farmers in this study indicated that farmers lacked basic pesticide knowledge. Label instructions generally do not cover mixtures of three or more pesticides and give no information on the compatibility or interaction of inert ingredients, such as emulsifiers and wetting agents, let alone the active ingredient. Mixing two different types of formulations, for example wettable powders with emulsible concentrates, is risky due to negative interactions. To substantiate this Smit et al. (2002) observed an interaction between fungicides, insecticides and water mineral content that reduced the efficacy of fungicides and insecticides in some tank mixtures. The refilling of knapsacks sprayers in the Surjan systems uses water from the channels which usually contains considerable suspended clay minerals that is likely to reduce chemical efficacy. Long-term use of chemical mixtures often results in the simultaneous development of resistance (Metcalf, 1980).

There is essentially no objective information available on the cross reaction and effects of the chemical mixtures identified in this study. Furthermore, most farmers did not consider that unspecified tank mixing of pesticides could be less efficacious and would cause adverse effects to their health or the environment. Tank mixing was carried out to save time, labour cost and with the anticipation of high efficacy in pest and disease control. The spraying volume applied ranged from 275 to 463 L ha^{-1} (Table 7.3). All farmers (100%) used only knapsack sprayers (of about 20 L volume) and no farmers used blower sprayers.

Table 7.2 Farmer knowledge and adherence to recommendations for safe pesticide use in shallot cropping in a survey of shallot farmer fertiliser practices in the Cirebon, Brebes, Bantul and Nganjuk regions of Java Indonesia in 2012.

	Cirebon (% Resp) (n=30)	Brebes (% Resp) (n=30)	Bantul (% Resp) (n=30)	Nganjuk (% Resp) (n=30)
Read label on the container before spraying				
Never	10.0	10.0	13.3	10.0
Sometimes	13.3	6.7	23.3	30.0
Always	76.7	83.3	63.3	60.0
Check the active ingredients on the label				
Never	10.0	36.7	40.0	30.0
Sometimes	16.7	16.7	16.7	33.3
Always	73.3	46.7	43.3	36.7
Uses pesticides based on particular target pest/disease				
Never	0.0	6.7	13.3	13.3
Sometimes	16.7	3.3	16.7	26.7
Always	83.3	90.0	70.0	60.0
Applies dosage as recommended on the label				
Never	23.3	23.3	26.7	30.0
Sometimes	46.7	50.0	53.3	43.3
Always	30.0	26.7	20.0	26.7
Mixes more than one chemical	100.0	93.3	86.7	90.0
Use of protective equipment				
None	80.0	53.3	13.3	43.3
Mask	20.0	16.7	66.7	56.7
Plastic coat – long sleeve shirt	0.0	30.0	10.0	0.0
Water proof gloves	0.0	0.0	10.0	0.0

Table 7.3 Farmer spray application volumes and timing in shallot cropping in a survey of shallot farmer fertiliser practices in the Cirebon, Brebes, Bantul and Nganjuk regions of Java Indonesia in 2012.

	Cirebon	Brebes	Bantul	Nganjuk
Average Spraying volume (l/ha)	370	463	275	347
First spraying (days after planting)	12.2	10.6	13.7	14.7
Last spraying (days after planting)	56.2	55.1	48.8	55.3

Most farmers (70-100%) carry out pesticide spraying early in the morning, while the rest (13-33%) apply pesticides late in the afternoon. In general, the first spraying of shallot is conducted at about 10-14 days after planting, shortly after shoot emergence. The first spraying for farmers in Brebes was 10.6 days after planting, while the last spraying was 55.1 days after planting. In Bantul, first spraying occurred 13.7 days after planting and the last spraying was applied at 48.8 days after planting. All farmers use knapsack sprayers to apply the pesticides. More than half of farmers, especially in Cirebon and Brebes do not adopt precautionary measures using fully body covers such as a mask, gloves and caps when using all chemicals. The use of mixed pesticides (cocktails) is quite common for most farmers in the four study sites. Overall excessive pesticide application is a major concern for farm labourer health and for food safety. There is an imperative to further scrutinise farmer pest management practices in shallot by identifying farmer awareness and perceptions on the health impacts to people applying pesticides, as well as the environment, and not just the contribution of pesticide use to reducing yield losses.

Figure 7.1. Chemical exposure to rural workers in Surjan vegetable farming systems in Java

The survey also assessed farmers' perceptions of the significance of 12 pests and diseases considered to be important in shallot cultivation. Farmers were asked to respond on a scale of 1 (very important) to 12 (not important). The results show that farmers in all the study regions consistently perceived Spodoptera (*Spodoptera exigua*) as the most important pest. However, the results across the study sites for other pests and diseases were less consistent. The four important pests/diseases for Cirebon were (1) Spodoptera exigua, (2) Peronospora destructor, (3) Alternaria porri, and (4) Fusarium sp. In Brebes the rankings were (1) Spodoptera exigua, (2) Colletotrichum spp., (3) Fusarium sp., and (4) Spodoptera litura. For Bantul, the rankings were (1) Spodoptera exigua, (2) Thrips tabaci, (3) Spodoptera litura, and (4) Liriomyza chinensis. In Nganjuk the rankings were (1) Spodoptera exigua, (2) Spodoptera litura, (3) Fusarium sp., and (4) Liriomyza chinensis, respectively. Farmers did not perceive that viruses had an

important impact on shallot crop productivity. Of particular interest was that downy mildew (*Peronospora destructor*) was identified as a serious disease in Cirebon since climatic conditions in Indonesian coastal zones would never favour downy mildew development suggesting a misdiagnosis of the underlying problem.

Socio-economic evaluation of shallot production and seed supply.

Baseline Survey for Improving Shallot Sustainable Productivity in Indonesia

The harvested area of shallot in Indonesia in the period of 2005-2014 showed an increasing trend. Annual shallot area fluctuated slightly over the last 10 years. The harvested area reached its peak of around 120,704 ha in 2014, after which the crop area has declined to about 93,667 ha in 2011. Total shallot production was approximately 732,609 t in 2005 and peaked at about 1.23 million t in 2014. During the period of 2010-2014, the volatility of shallot yield was quite low, implying that the productivity of shallot was quite stable, with the average annual yield of 9.4 t/ha.

The ten largest shallot producing provinces in Indonesia are North Sumatra, West Sumatra, West Java, Central Java, Yogyakarta, East Java, West Nusa Tenggara, East Nusa Tenggara, Central Sulawesi, and South Sulawesi. Java Island contributes about 70% of the harvested area, and 77% of national shallot production. In 2014, Central Java contributed the highest share of the national shallot production with 38.3% of the harvested area and 42.1% of the tonnage. Central and West Java had the highest yields.

During the period of 1981-2014, shallot consumption tended to increase with an average growth of 8.7% per year. Shallot consumption in 1981 was 1.65 kg/person/year and increased to 2.49 kg/person/year in 2014. The highest consumption of shallot occurred in 2007 and reached 3.01 kg/person/year.

In the period of 1996-2014, the volume of shallot imported was consistently higher than the volume of shallot exported. In 1996, the export volume was 7,171 t and decreased to 4,439 t in 2014. The highest export was in 2012 (18,754 t), while the lowest export occurred in 1998 (176 t). Meanwhile, in 1996, the import volume was 42,057 t and increased to 74,903 t in 2014. The highest import volume was in 2011 at 160,467 t. Minister of Agriculture Regulation no. 60/Permentan/OT.140/9/2012 has shown an effect in reducing importation by 22.1% from 2012 (120,354 t) to 2013 (93,737 t). The value of shallot export increased during the period of 1996-2014. In 1996, the value of shallot exports was USD1.62 million and in 2014 was USD2.98 million; a more than threefold increase. Export-import data from 1996 to 2014 suggested that Indonesia is a net importer of shallot since, during that period, the value of export was consistently lower than the value of imports. There has been a continuing increase in import value of shallot from 1996 (USD 15.6 million) to 2014 (USD 28.31 million). The highest import value was USD 77.4 million in 2011.

One of the major problems facing shallot growers is price fluctuation. The price of shallot becomes more volatile in response to greater fluctuations in supply and inelastic demand for shallot with respect to price. The considerable shallot price fluctuation may be due to

(a) climatic conditions that affect the quantity and quality of shallot, hence influences the general price level;
(b) inadequate storage facilities that could not be used for longer storage to level-off price fluctuation.
(c) strong suspicion that oligopolistic practices exist in the shallot marketing – big speculative traders with vast financial resources are collaborating to exert shallot supply and shallot price level.

In general, market prices for shallot follow a seasonal pattern where the lowest

corresponds to peak harvest in August-September, and are highest between February and June when domestic supply is lowest.

Across the four study sites the main shallot cultivars grown included Bima Curut, Timur, Illocos, Biru, Tiron, Philip and Thailand. In Brebes, the main shallot variety chosen by all farmers for their July-September crop was Bima Curut, which is also popular in Cirebon where it was planted by more than 60% of respondents. Some farmers in Cirebon also used alternative varieties, such as Timur and Illocos (imported). Biru is the most preferred variety used by farmers in Bantul along with Tiron as the second preferred cultivar. Meanwhile, the only variety used by farmers surveyed in Nganjuk for their last crop was the imported variety Thailand. In all sites, farmers' familiarity with IVEGRI's varieties was low, except for Bima and Kuning. Though IVEGRI is not acknowledged for this, these two varieties were the result of purification of two local varieties from Brebes and Tegal areas. The suitability and superiority of agronomic characteristics, along with high yield potential and good quality bulbs, are consistently considered by respondents in all locations as the most important factors influencing a farmer's decision to select a shallot variety.

The majority of farmers (particularly in Brebes, Bantul and Nganjuk) indicated their principal seed source is their own saved seed. In Nganjuk, almost 100% of surveyed farmers retained their own shallot bulbs for seed. In Cirebon, almost half of the respondents indicated that they plant seed purchased from other farmers, mainly from Brebes. These data are reflective of a seed system in which the majority of farmers mostly source their seed from informal channels that include combinations of farm saved seeds, seed exchanges among farmers and local shallot bulb-seed markets. This system contributes about 95-100% of shallot seed supply.

The average total costs of shallot cultivation in the four study sites ranged between 50-70,000,000 IDR/ha (~AUD 5-7,000/ha). The highest cost was labour which accounted for 38-48% of the total costs. The second highest cost was shallot seed which accounted for 30-36% of the total costs. The cost for pesticides was comparable across the four regions at about 5-9% of the total cost and fertiliser accounted for 6-10% of the total cropping cost. Based on the average yield and total costs, the production costs of shallot was about 3,000-4,700 IDR/kg.

In Cirebon, the three most important constraints to shallot production as perceived by farmers were (1) incidence of pests and diseases, (2) soil fertility, and (3) availability of irrigation water. Constraints that were ranked as the three most important in Brebes were (1) soil fertility, (2) incidence of pests and diseases, and (3) soil type. In Bantul, the most important constraint was soil fertility, and followed by incidences of pests and diseases and availability of irrigation water, respectively. Meanwhile, the most critical constraint to production in Nganjuk was incidence of pests and diseases, followed by soil fertility and potential yield, respectively.

Analysis of Shallot Supply Chain in Java.

The survey results reveal that most problems encountered in the shallot supply chain are similar across the four survey districts of Cirebon, Brebes, Yogyakarta and Nganjuk. The identified strengths, weaknesses, opportunities, and threats of the four shallot supply chains in the four study sites are as follows.

Strengths:

- Shallot supply chains in Cirebon, Brebes, Yogyakarta and Nganjuk are operating under the forces of supply and demand.
- The coefficient of variation of shallot weekly prices is lower compared with the coefficient of variation of other vegetables weekly prices.

- There is moderate to strong market integration among various shallot markets in Cirebon, Brebes, Yogyakarta and Nganjuk.
- Quite large number of buyers and sellers are involved in the shallot transaction suggesting there is no monopoly or monopsony practice in the shallot supply chain in Cirebon, Brebes, Yogyakarta and Nganjuk.
- Most traders buy and sell shallot based on differential product quality or class (mostly size-based grading).

Weaknesses:
- Shallot supply chains are basically still dominated by the traditional chain in which the main outlet is the traditional markets.
- There are high storage losses due to the initial physical condition of harvested shallot, storage conditions, storage time duration, and pre-stored inappropriate treatments.
- Regular business record-keeping is still not practiced by most supply chain participants.
- There is very little innovation within the shallot supply chain and very few participants seek technical and market information that would allow them to reduce costs, increase profitability and exploit new market opportunities.
- Some supply chain participants, especially retail traders, operate on small-scale business and lack specialization.
- Poor flow of information leads to higher than necessary transaction costs.

Opportunities:
- Existing conditions of production agro-ecosystems still provide some possibilities to increase the yield of good quality shallots.
- The geographical position of Cirebon, Brebes, Yogyakarta and Nganjuk production centers allow shallot to not only be marketed to meet local needs, but also to meet regional and even export demands.
- The complexity of the existing shallot supply chain is still relatively low which may allow some improvements in marketing efficiency.

Threats:
- Limited availability of market information tends to lead to distrust among shallot supply chain participants.
- The risk of supply shortages commonly experienced by all chain participants during the wet season or when crop failures occurred.
- Liquidity problems that may severely limit the efforts to achieve business economies of scale.
- Competition from exported shallots with cheaper price.

Shallot farmers in Indonesia face a similar cost–price squeeze that affects farmers in other countries. The need to feed rapidly growing populations tends to result in policies that keep food prices low, making innovation and investment unattractive, even if the necessary capital and management capacities are available. However, there are opportunities for farmers in response to a growing consumer class based on an expanding total population and increasing urban population. In the short term, this group can be expected to spend more on higher shallot quality and more varied produce than do people at lower income levels. Projections on future trends suggest that, in terms of volume, most of the growth in demand for food will be in middle-class consumption. Much of this demand will be met domestically but against competition from other exporting countries (Thailand, India, and Vietnam). The primary basis of competition will

be price but the presence of exporting competitors will also mean the gradual adoption of 'world' quality standards. This effect is enhanced by the increasing implications of the WTO for developing countries. For this reason, there is no option for Indonesia but to accelerate the strengthening of competitive capacity. In helping farmers take advantage of the growth opportunity to supply the consuming classes, the aim should be to build the capacity of domestic producers to match the products that exporting countries will be aiming to put into Asian markets. The priority to increase the competitiveness of domestic agriculture and agribusiness is also set by the Indonesian Ministry of Agriculture (MOA 2001). This represents an incentive for improving supply chain management (SCM) in Indonesian shallot. In summary, some proposed interventions are grouped to comply with the key principles of successful shallot supply chain management (Woods 2004):

Shallot Seed System in Cirebon, West Java; Brebes, Central Java; Bantul, Yogyakarta; and Nganjuk, East Java.

The limited role of a formal seed system has made the shallot sub-sector heavily dependent on an informal seed supply system. The informal system is dominant for several key reasons including

(1) It is relatively cheaper and readily available in the farmer's villages just at the time the seed is needed.
(2) It allows farmers to observe the shallot seed crop production in the field before deciding to buy.
(3) It is more reliable and its sustainability is more guaranteed than seed from a formal system.

In the informal seed system, farmers use their-own strategies, including the improvement of farmer-saved seed, farmer-to-farmer seed exchange, and farmer-managed seed production. However, seed provided through this chain has limitations in terms of quality of planting material, diseases, virus and consequently low yield. In general, the shallot seed supply system is still characterized by low-quality seed, limited clean and healthy seed, lack of supporting qualified and professional human resources, lack of supporting infra-structure, and low transfer technology of seed production to both farmers and seed growers.

In order to help strengthen the shallot seed system in Indonesia, the six principles of a sustainable seed system should be adopted including.

(1) cost-recovery - the ability of the 'system' to recover the cost of producing, multiplying and distributing seed.
(2) Quality - the ability of the 'system' to supply quality seed to farmers.
(3) Quantity – the ability to supply enough quantity of quality seed to meet the demand
(4) Diversity - the ability of the 'system' to supply adequate quantity and quality of diverse varieties of seed to meet the demand
(5) Service/accessibility - the ability to deliver the seed in a timely manner in locations that are accessible to farmers.
(6) Price - the ability of the 'system' to supply the seed at an affordable price.

Although no systematic information on the informal shallot seed supply is available, the focus group discussion confirms the importance and potential of local seed systems for a diverse, flexible and readily available seed supply for small farmers. In remote and heterogeneous areas, this system is still more important and efficient than the formally organized seed systems. Farmers' seed production, handling and selection practices are often well-developed, and seed exchange mechanisms can often adequately supply seed. It is also increasingly recognized that farmers' knowledge and capacity to locally produce and maintain genetic material plays an important role in the continued

improvement in crop productivity. Because of these valuable characteristics of the informal seed supply system and its adaptation to local conditions and preferences, it is suggested that shallot seed supply for small farmers is best improved through the strengthening of this local system, rather than by replacing it with seed supply through a formal system.

Furthermore, it is suggested that a strengthening of informal shallot seed supply systems is best achieved through the development of linkages between this system and formal seed supply systems to become an integrated shallot seed supply system. It may be expected that a strengthening of the informal seed systems will at the same time greatly improve the performance of the formal seed supply systems by alleviating the difficulty of supplying small and remote farmers, representing diverse and variable seed markets. The formal system could organise the distribution of new cultivars and the supply of good quality seed to small farmers to a large extent through the informal seed system, using and developing knowledge and expertise available in this system. The formal system can then concentrate on tasks and activities for which it is best equipped: breeding and maintenance of a wide range of material and production of small volumes of good quality seed.

A formal seed system that functions well and adequately supports the informal system with material and technical assistance is complementary and mutually dependent. Involving farmer participation in breeding, improved local seed production and distribution are promising perspectives for an integrated approach to improving seed supply. However, it requires the informal seed system to be recognised as a rational system, complementary to the formal one and that farmers are not only considered as clients, but also as participators in variety development and seed supply.

7.2 Distribution, diagnostics and management of pathogens in Indonesia and Australia

7.2.1 Survey of shallot and chilli virus incidence across Indonesia

Incidence of virus infecting shallot

Field assessments of crop health and symptoms identified the most prevalent virus symptoms to be yellow and yellow striped leaves, and wrinkled leaves and often combinations of various symptoms was identified as previously by Gunaeni *et al*. (2011). Across the surveyed districts the range of shallot varieties and farmer agronomic practices varied greatly (Table 7.4) and in general farmers obtain seed from their own crops. The main insect pest in most regions was *Spodoptera exigua*, while the main disease was fusarium wilt caused by *Fusarium oxysporum*.

Table 7.4 General condition of shallots fields in several production centers.

Variables	Shallot production regions				
	Brebes	Probolinggo	Bima	Solok	Enrekang
Elevation (asl)	7 - 10 m	4 - 47 m	15 -25 m	1496- 1556 m	426- 883 m
Variety	Bima Curut	Biru Lancur	Super Philips	Alahan Panjang (local)	Kapur (Katumi)
Plant age (DAP)	40 - 42	30 - 35	30 - 35	35 - 40	31 - 40

Source of seedbulb	Own sources	Own sources	Own sources	Own sources	Buy from seed growers
Plant spacing	15 x 20 cm	18 x 20 cm	varies	20 x 20 cm	20 x 20 cm
Organic materials	None	Less	Enough	Enough	Less
Major weed	-	grasses	-	-	grasses
Major pests	*S.exigua*	*S. exigua*	*S. exigua*	*S.exigua* *Lyriomiza* sp.	*S. exigua*
Major diseases	Fusarium wilt	Fusarium wilt	Fusarium wilt	-	Fusarium wilt
Pests & disease control	Pesticide	Pesticide	Pesticide	Pesticide	Pesticide
Productivity (ton/ha)	8-9	9 - 10	11-12	13 - 15	9-10

Virus detection from field samples using the DIBA method confirmed the presence of OYDV, SYSV, SLV and GarCLV infections. The four main viruses were found in all locations, and the highest virus incidence (100%) occurred in Enrekang, Bali, and Lombok (Fig 7.2). Virus infection in the field was high but the majority of farmers failed to recognize the problem due to their unfamiliarity with the symptoms and the potential impact on yield. The four main viruses were found in all locations, except in Bima where only SLV and SYSV were found (Fig 7.2). These data confirmed the accumulation of virus infection in the bulbs.

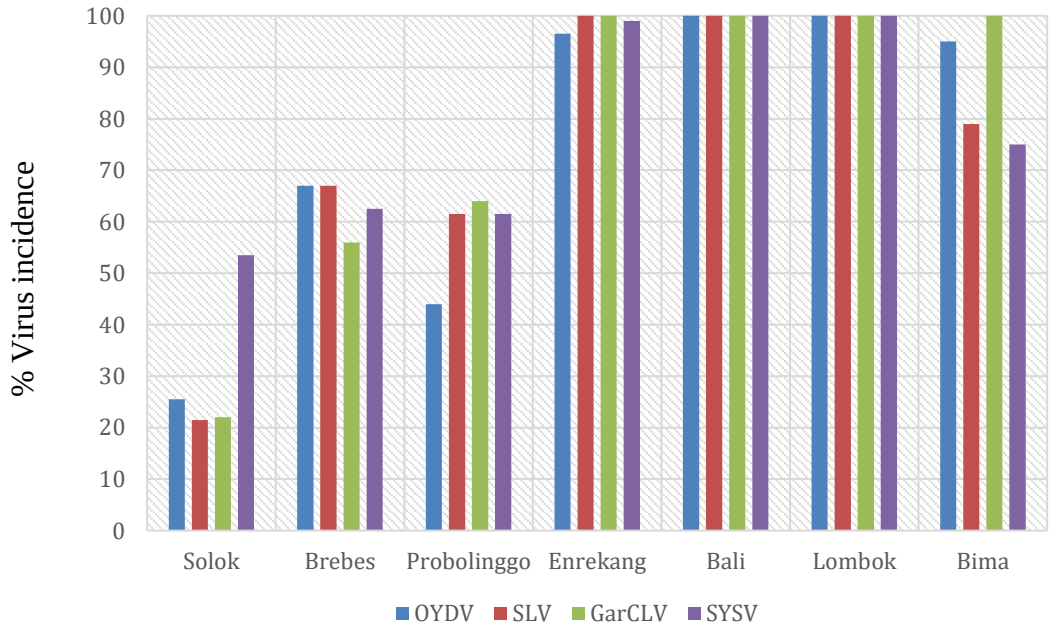


Figure 7.2. Incidence of major viruses infecting shallot on the field based on detection of leaf samples using specific antibodies

Incidence of virus infecting chilli pepper

High incidence of PYLCV (>60%) was recorded at all sites with up to 100% infection recorded at Bali Lombok and Bima. General symptoms commonly found in the field, involved yellow and green mosaic, mottle, leaf curling and cupping, and also plant dwarfing. Based on virus detection in the laboratory, PYLCV was the most dominant virus in all locations, followed by CMV and ChiVMV (Fig 7.3).

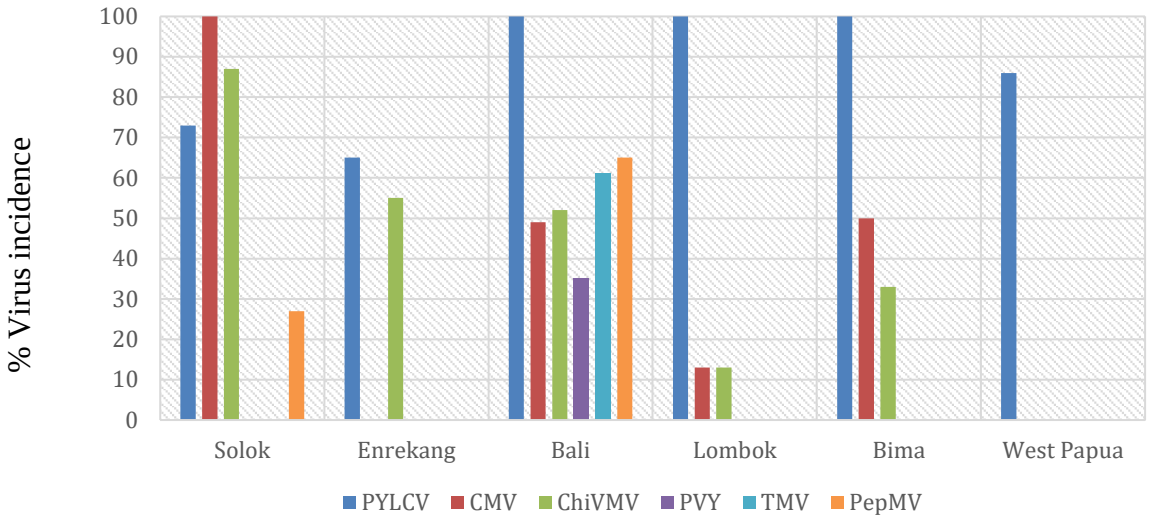


Figure 7.3. Incidence of major viruses infecting chillipepper on the field based on detection of leaf samples using specific antibodies (CMV, ChiVMV, PVY, TMV, and PepMV) and universal primer of *Begomovirus* for PYLCV

7.2.2 Survey results for virus diversity and distribution in Australian garlic

The collected samples were tested for the three potyviruses, OYDV, SYSV and LYSV, three carlaviruses, GCLV, GLV and SLV, and five allexiviruses, GVA, -B, -C, and –X and ShVX. Virus incidence within planting material was near 100%, with plants carrying at least one virus from each of the genera tested (Poty, Carla, and Allexi). The most common infections were by the potyviruses, OYDV and LYSV, and the carlaviruses, SLV and GCLV. Plants were also infected by a varied profile of Allexiviruses, most commonly; GV-A, -B, and X. Details of specific survey sites are listed in Table 7.5 and 7.6. The commercial availability of virus-free seed stocks in Victoria and Tasmania may have contributed to differences in virus incidence among garlic growing regions in Australia.

Table 7.5. Details of field surveys of Australian garlic. For QLD surveys, all samples were tested individually and for the remaining surveys, the 300 individual samples were bulked into groups of ten and the thirty bulked samples tested.

Site	Date	Location	Number tested
WP285	2013	Thornton, QLD	238 ˣ
WP286	2013	Lower Tent hill, QLD	94 ˣ
WP287	2013	Kalbar, QLD	90 ˣ
WP288	2013	Mt Sylvia, QLD	94 ˣ
VIC site 1	2015	East Gippsland, VIC	300 ˣˣ
VIC site 2	2015	East Gippsland, VIC	300 ˣˣ
VIC site 3	2015	Swan Hill, VIC	300 ˣˣ
VIC site 4	2015	Merbein, VIC	300 ˣˣ
VIC site 5	2015	Merbein, VIC	300 ˣˣ
VIC site 6	2015	Mildura, VIC	300 ˣˣ
VIC site 7	2015	Mildura, VIC	300 ˣˣ
VIC site 8	2015	Daylesford, VIC	300 ˣˣ
NSW site 9	2015	Buronga, NSW	300 ˣˣ
SA site 10	2015	Renmark, SA	300 ˣˣ
ˣ Individual samples ˣˣ Samples bulked in lots of 10 for molecular indexing.			

Table 7.6. Species-specific virus detection in Australian garlic.

	Allexivirus (%) infected						Carlavirus (%) infected				Potyvirus (%) infected			
Survey Site	Genus[1]	GVA[2]	GVB[2]	GVC[2]	GVX[2]	ShVX[2]	Genus[1]	GCLV[2]	GLV[2]	SLV[2]	Genus[1]	OYDV[2]	SYSV[2]	LYSV[2]
WP285 QLD	239/238	66	63	3	80	0	237/238	3	0	100	237/238	98	0	98
WP286 QLD	93/94	66	94	30	63	0	93/94	0	0	90	93/94	95	0	88
WP287 QLD	89/90	52	91	0	30	0	90/90	99	0	100	82/90	99	0	100
WP288 QLD	88/94	84	93	3	62	0	93/94	89	0	81	86/94	67	0	78
Site 1 VIC	30/30	>29 (19.4-100)[3]	0 (0-1.2)	0.5 (0.08-1.9)	29 (16.1-51)	0 (0-1.2)	30/30	14 (8.2-20.6)	0 (0-1.2)	>29 (19.4-100)	29/30	21 (12.4-32)	0 (0-1.2)	4 (1.9-7.2)
Site 2 VIC	28/30	0 (0-1.2)	20.6 (12.4-32)	3 (1.04-5.3)	0 (0-1.2)	0 (0-1.2)	28/30	11 (6.8-17.4)	0 (0-1.2)	16 (10-25)	27/30	>29 (19.4-100)	0 (0-1.2)	15 (9.1-22.6)
Site 3 VIC	30/30	0 (0-1.2)	15 (9.1-22.6)	1 (0.08-1.9)	>29 (19.4-100)	0 (0-1.2)	30/30	>29 (19.4-100)	0 (0-1.2)	>29 (19.4-100)	30/30	>29 (19.4-100)	0 (0-1.2)	9 (5.1-13.8)
Site 4 VIC	30/30	0 (0-1.2)	18 (11.1-28)	1 (0.2-3.03)	>29 (19.4-100)	0 (0-1.2)	30/30	>29 (19.4-100)	0 (0-1.2)	>29 (19.4-100)	29/30	16 (10-25)	0 (0-1.2)	0 (0-1.2)
Site 5 VIC	29/30	0 (0-1.2)	21 (12.4-32)	0.5 (0.08-1.9)	29 (16.1-51)	0 (0-1.2)	30/30	4 (1.6-6.6)	0 (0-1.2)	>29 (19.4-100)	29/30	24 (14-38.1)	0 (0-1.2)	4 (1.9-7.2)
Site 6 VIC	25/30	0 (0-1.2)	15 (9.1-22.6)	0.5 (0.01-1.9)	14 (8.2-20.6)	0 (0-1.2)	30/30	29 (16.1-51)	0 (0-1.2)	>29 (19.4-100)	30/30	29 (16.1-51)	0 (0-1.2)	5 (2.5-8.6)
Site 7 NSW	30/30	0 (0-1.2)	16 (10-25)	0 (0-1.2)	0.5 (0.01-1.9)	0 (0-1.2)	30/30	18 (11.1-28)	0 (0-1.2)	>29 (19.4-100)	30/30	>29 (19.4-100)	0 (0-1.2)	4 (1.6-6.6)
Site 8 VIC	29/30	0 (0-1.2)	21 (12.4-32)	0 (0-1.2)	29 (16.1-51)	0 (0-1.2)	30/30	>29 (19.4-100)	0 (0-1.2)	>29 (19.4-100)	29/30	29 (16.1-51)	0 (0-1.2)	2 (0.8-4.8)
Site 9 SA	(12/30)	0 (0-1.2)	0 (0-1.2)	0 (0-1.2)	4 (2.2-7.9)	0 (0-1.2)	30/30	0 (0-1.2)	0 (0-1.2)	>29 (19.4-100)	30/30	29 (16.1-51)	0 (0-1.2)	2 (0.6-4.2)
Site 10 VIC	29/30	2.2 (0.8-4.8)	0 (0-1.2)	15 (9.1-22.6)	18 (11.1-28)	0 (0-1.2)	30/30	3 (1.3-6)	0 (0-1.2)	>29 (19.4-100)	29/30	29 (16.1-51)	0 (0-1.2)	18 (11.1-28)

[1]Determined using genus-specific primers in RT-PCR
[2]Determined in a hybridisation assay using labelled probe, which was developed using species-specific primers
[3]95% confidence limits

Bulked samples

In specific evaluations of yield and bulb size in a Queensland crop, cloves from individual bulbs had the same virus genus complement when tested with the generic PCR, and all bulbs were similarly infected regardless of bulb size. Evaluation of individual virus species showed minor variation between cloves from individual bulbs using probe assays. The individual virus species profile was mostly uniform across the two planting material types (Table 7.7).

Table 7.7. Effects of virus on yield and bulb size in Queensland garlic.

	Large	Small
Number of bulbs	7	14
Average bulb weight (g)	86.6	24.7
Average number of cloves per bulb	16.1	7.2
Average clove weight (g)	6.0	3.3
Average germination rate of cloves (%)	97.3	93.1
% plants with rating 1	0.1	1.1
% plants with rating 2	81.5	81.5
% plants with rating 3	17.6	17.4
% plants with allexivirus	100	100
% plants with carlavirus	100	100
% plants with potyvirus	100	100

Large-scale evaluation of virus diversity in garlic isolates across Australia, and overseas, provides valuable information concerning the geographic origin of germplasm used in the industry, as well as allowing for tracing the movement of garlic and its pathogens along trade routes. Because the allexivirus GVC has not yet been identified in China, the presence of this virus in Australian crops could indicate that planting material did not originate in China. In the study, the incidence of GVC was not widespread among the surveyed sampled, however, there were pockets of infection existing throughout QLD, and at one location in Victoria. Assessment of diversity among virus populations across Australia and their insect vectors and potential weed hosts, contributes to our understanding of virus epidemiology and supports quarantine efforts designed to limit the entry and spread of new viruses into Australia. In addition, this survey supports the need for further development of commercially viable virus free garlic germplasm program in Australia as chronic virus infection is knon to reduce yields and contributes to targeted pest management strategies within the garlic industry.

7.2.3 Fusarium disease complex in Indonesian shallot

Survey of Fusarium incidence in Java

The result of this study showed that the shallot cultivars Thailand and Bauji were widely planted in Nganjuk district, whereas in Bantul district the main cultivars were Biru and Tiron. In the dry season, Moler disease incidence for all four shallot cultivars was quite low at 10% for cv. Thailand, 7.0% for Bauji, 2.0% for Biru and 2.0% for Tiron (Table 7.8). In the wet season, cvs. Thailand and Biru were harvested at less than eight weeks after planting due to the rapid development of Moler disease in these cultivars, so that the farmers harvested the shallot early to avoid crop failure. However, for cv. Bauji, disease incidence was quite low (8%) until the end of the observation period, whereas for cv. Tiron the disease incidence was quite high (36%) (Table 7.8).

Table 7.8. Moler disease incidence of five shallot cultivars in wet and dry season in Nganjuk, Bantul, Brebes and Cirebon.

Districts	Shallot cultivars	Moler disease incidence (%)	
		Dry Season	Wet season
Nganjuk	Thailand	10.0 a	0.0c
Nganjuk	Bauji	7.0 ab	8.0 b
Bantul	Tiron	2.0 bc	36.0 a
Bantul	Biru	2.0 bc	0.0c
Brebes	Bima curut	8.7%	2.7%
Cirebon	Bima curut	1.3%	4.6%

Identification of Fusarium spp. the causal agent of Moler disease

A survey of the incidence of Moler disease was conducted in Batu and Nganjuk (East Java), Kulon Progo, Bantul, Gunung Kidul, Sleman (Yogyakarta), Temanggung, Pemalang and Brebes (Central Java), Cirebon (West Java), and Enrekang (South Sulawesi) districts. Isolations from Moler disease symptomatic plants resulted in the identification of 44 isolates of *Fusarium* spp. The result of pathogenicity test (Table 7.9) showed that of 44 the *Fusarium* spp. isolates, all of them were able to cause Moler disease (Fig. 7.4) on shallot plants with the percentage of disease incidence ranged from 11.1% to 100%. Identification using ITS primers showed that from 44 isolates, 33 isolates were *Fusarium solani*, 8 isolates were *F. acutatum* and 3 isolates were *F. oxysporum*.

Table 7.9. Disease symptoms and disease incidence (Dis_Inc %) caused by Fusarium spp. isolated from shallot

No	Isolates	Species	Symptom	Dis_Inc (%)	No	Isolates	Species	Symptom	Dis_Inc (%)
1	BntP	*F. solani*	Wilting	22.2bc	23	SkmBP	*F. acutatum*	Bulb rot	66.6bc
2	BntB	*F. solani*	Wilting	33.3bc	24	GndM	*F. solani*	Bulb rot	22.2bc
3	B3	*F. solani*	Wilting	22.2bc	25	GndBP	*F. solani*	Bulb rot	11.1c
4	Mda	*F. acutatum*	Wilting	44.4bc	26	KP2	*F. acutatum*	Bulb rot	11.1c
5	Mdb	*F. solani*	Wilting	100.0a	27	KP3	*F. solani*	Bulb rot	22.2bc
6	Prc	*F. acutatum*	Wilting	44.4bc	28	T6	*F. solani*	Bulb rot	11.1c
7	SMS 3.2	*F. solani*	Wilting	22.2bc	29	T1R	*F. solani*	Bulb rot	11.1c
8	Bnd	*F. solani*	Wilting	33.3bc	30	KP4Bm	*F. solani*	Bulb rot	22.2bc
9	Bre	*F. solani*	Wilting	44.4bc	31	ERKSS	*F. solani*	Bulb rot	33.3bc
10	Brf	*F. solani*	Wilting	22.2bc	32	SkmBB	*F. solani*	Moler disease	11.1c
11	KsnBC	*F. solani*	Wilting	22.2bc	33	CRB1	*F. oxysporum*	Moler disease	22.2bc
12	Ngg	*F. solani*	Wilting	55.5bc	34	CRB2	*F. solani*	Moler disease	44.4bc
13	SKMT	*F. solani*	Wilting	44.4bc	35	CRB3	*F. solani*	Moler disease	22.2bc
14	CRB4	*F. solani*	Wilting	11.1c	36	PbdBC	*F. solani*	Moler disease	33.3bc
15	BRT 2.2	*F. oxysporum*	Wilting	66.6bc	37	KP1	*F. solani*	Moler disease	11.1c
16	T8	*F. acutatum*	Wilting	44.4bc	38	T3	*F. oxysporum*	Moler disease	44.4bc
17	KP4T	*F. solani*	Wilting	11.1c	39	KP4B	*F. solani*	Moler disease	22.2bc
18	Trgj	*F. solani*	Wilting	22.2bc	40	SygGK	*F. solani*	Moler disease	44.4ab
19	BntK	*F. solani*	Bulb rot	11.1c	41	KrgGK	*F. solani*	Moler disease	11.1c
20	SMS 3.1	*F. solani*	Bulb rot	22.2bc	42	KrsGK	*F. solani*	Moler disease	22.2bc
21	KsnBW	*F. acutatum*	Bulb rot	22.2bc	43	BgrGK	*F. solani*	Moler disease	11.1c
22	Ngh	*F. acutatum*	Bulb rot	11.1c	44	LngBm	*F. acutatum*	Moler disease	11.1c

Figure 7.4. Disease symptom caused by *Fusarium* spp. isolated shallot showing Moler disease: (a) healthy plant; (b) wilting; (c) bulb rot; (d) twisted.

Shallot cultivar sensitivity to fusarium infection

A series of 16 commercial shallot cultivars were screened for resistance to Moler disease (Table 7.10). The screening identified cultivar Sembrani as resistant (5.1% incidence) whilst highly susceptible cultivars were Markonah and Majun (~29% incidence) and other cultivars expressed intermediate sensitivity to Moler disease.

Table 7.10. Response of 23 shallot cultivars towards Moler disease.

No.	Shallot cultivars	Moler disease incidence (%)	Category
1	Bima curut	18.9	moderate
2	Timur Carwan	24.7	moderate
3	Markonah	28.8	susceptible
4	Kuning	19.6	moderate
5	Maja Cipanas	18.7	moderate
6	Pikatan	18.9	moderate
7	Kramat-1	17.8	moderate
8	Katumi	22.4	moderate
9	Trisula	12.2	moderate
10	Kramat-2	18.6	moderate
11	Pancasona	21.7	moderate
12	Majun	28.6	susceptible
13	Lembah Palu	11	moderate
14	Sembrani	5.1	resistent
15	Sipayung	22.8	moderate
16	Mentes	24.6	moderate

Effect of shallot seed-bulb treatment on fusarium.

A seed treatment experiment was conducted to evaluate the impact on fusarium incidence over four cultivars (cvs. Kuning, Trisula, Crok and Tiron). The control treatments included, submerging bulbs in aquadust as a positive control for *Fusarium* sp. inoculation (P1), the negative control was without *Fusarium* sp. inoculation (P2). The other treatments included

submerging bulbs in hot water at 45°C for 15 min (P3), 45°C for 30 min (P4), 50°C for 15 min (P5) and at 50°C for 30 min (P6), bulbs immersed in fungicide (P7), and a treatment of bulbs immersed in diluted biofertilizer (P8). Kuning was the most susceptible cultivar to fusarium infection followed by Crok, and Trisula respectively (Table 7.11). Tiron exhibited no infection across all treatments. The most effective heat treatment for minimising fusarium was 45°C for 30 minutes and heat treatments of 45°C did not affect shallot plant growth but treatments at 50°C tended to reduce plant bulb yield (Table 7.12).

Table 7.11. Moler disease incubation period on 4 shallot cultivars by various seed treatments.

Treatment	Disease Incidence (%)			
	Tiron (B1)	Crok (B2)	Trisula (B3)	Kuning (B4)
P1	0.0	33.3	0.0	100.0
P2	0.0	33.3	100.0	100.0
P3	0.0	66.7	0.0	0.0
P4	0.0	33.3	66.7	33.3
P5	0.0	0.0	66.7	0.0
P6	0.0	33.3	0.0	0.0
P7	0.0	0.0	66.7	66.7
P8	0.0	0.0	0.0	100.0

Table 7.12. Fresh weight of four shallot cultivars after treated with various seed treatments.

Treatment	Shallot fresh weight (grams)							
	Tiron (B1)		Crok (B2)		Trisula (B3)		Kuning (B4)	
P1	17.5	abcdef	23.3	abcd	16.6	abcdef	18.8	abcdef
P2	11.6	bcdef	25.9	abc	17.9	abcdef	23.6	abcd
P3	16.1	abcdef	34.5	a	22.1	abcde	5.5	def
P4	17.4	abcdef	17.3	abcdef	27.6	ab	15.2	abcdef
P5	5.7	def	11.6	bcdef	14.9	bcdef	6.8	cdef
P6	5.0	def	9.2	bcdef	4.7	def	1.6	f
P7	2.8	ef	5.4	def	12.9	bcdef	6.0	def
P8	6.6	cdef	5.5	def	5.5	def	3.0	ef
Average	10.4		16.6		15.3		10.0	

Effect of nitrogen rate on shallot fusraium incidence.

An experiment was conducted on the effect of nitrogen rate (0 (N0), 50 (N1), 100 (N2), 150 (N3) and 200 (N4) kg N ha^{-1} as urea) on the impact of fusarium in shallot grown in a sterilised soil inoculated and non-inoculated. In sterile soil, there was no fusarium incidence and in the inoculated soil incidence of fusarium was highest in the 0N treatment and decreased at an optimised N rate of 100 kg N ha^{-1} (Table 7.13). A further increase in N application rate of 200 kg N ha^{-1} increased fusarium incidence. The addition of urea fertilizer in sterile soil treatment did not stimulate the occurrence of Moler disease in shallot, and, in inoculated soil the addition of urea fertilizer at the recommended rate (100 kg N ha^{-1}) suppressed Moler disease in shallot giving 0% infection. In the sterilised soil there was no response to N (Fig. 7.5) presumably due to a high basal soil N level but under inoculation and low N fusarium incidence was

greatest. Since there was no absolute N response the data suggests that the application of N at an optimised level suppressed fusarium in shallot.

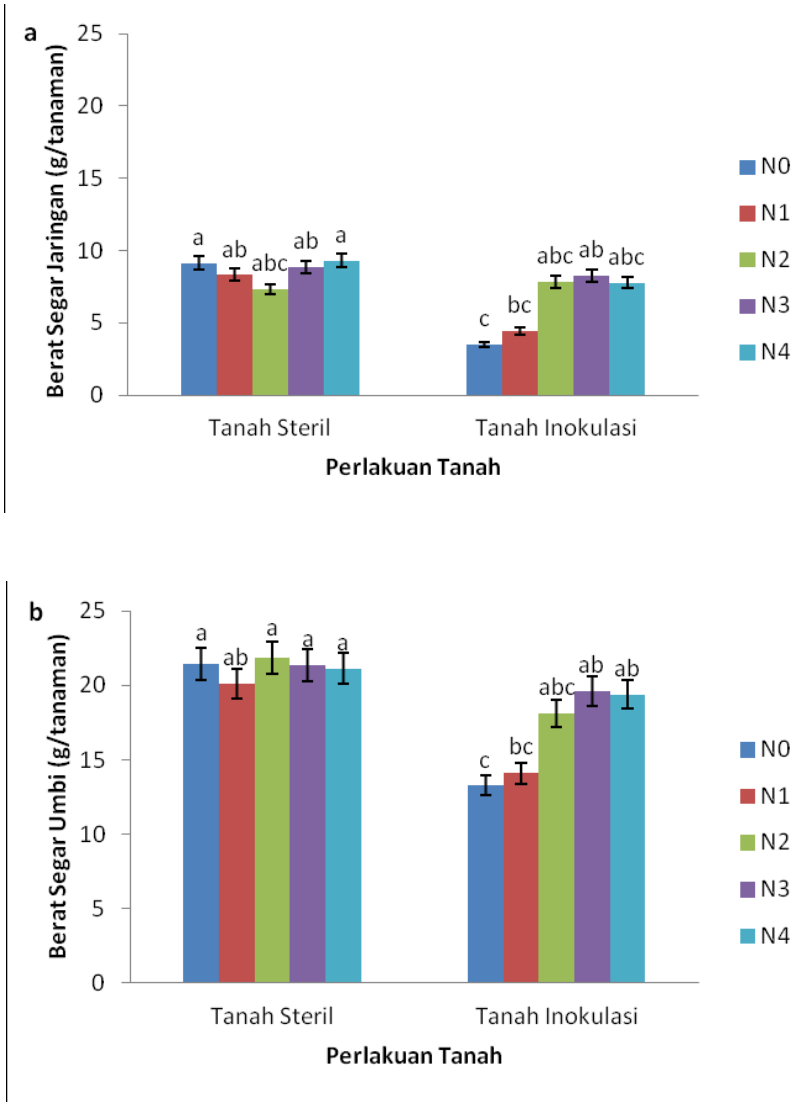


Figure 7.5. Effect of urea fertilizer application in sterile and Fusarium inoculated soil on fresh weight of shallot a) shoot and b) bulb

Table 7.13. Effect of urea fertilizer application on the occurrence of shallot Moler disease in sterile and Fusarium inoculated soil

Treatment	Moler disease incidence (%)	
	Sterile soil	Fusarium inoculated soil
N0	0 b	37.5 a
N1	0 b	25.0 ab
N2	0 b	0.0 b
N3	0 b	12.5 ab
N4	0 b	25 ab
Average	0	20

Selection of shallot rhizobacteria for controlling Moler disease

The results of this study showed that there were some beneficial rhizobacteria isolated from shallot's rhizosphere, which could be used to control Moler disease on shallot and included *Bulkholderia* and *Bacillus* (Table 7.14). The isolate BrSG.3 is closely related to *Burkholderia seminalis*, isolate BrSG.5 and Bp25.6 are closely related to *Bacillus amyloliquofaciens*, isolate BrSM.4 is closely related to *Burkholderia cepacia* strain NTUIOB TPC6, isolate Bp25. 2 is closely related to *Bacillus methylothrophicus* and isolate Bp25.7 is closely related to *Bacillus subtilis*. The *Bacillus* spp. isolates show strong potential for biological control of Moler disease in shallot and increased plant growth.

Table 7.14. Inhibition percentage of antagonistic rhizobacteria against *Fusarium acutatum* and hypersensitive reaction test (HS test)

No	Bacterial strains	Disease Inhibition (%)	HS test	No	Bacterial strains	Disease Inhibition (%)	HS test
1.	Ph.1	39.9	-	18.	Bp25.6	65.4	-
2.	Ph.2	43.7	-	19.	Bp25.7	59.1	-
3.	Ph.3	42.4	-	20.	Bp25.8	41.4	-
4.	Ph.5	39.3	-	21.	Bp25.9	49.6	-
5.	Ph.12	42.1	-	22.	Bp25.11	47.9	-
6.	BrSM.2	39.6	+	23.	Bp55.4	48.7	-
7.	BrSM.4	50.0	-	24.	Bp55.6	56.4	-
8.	BrSM.10	39.6	-	25.	Bbs25.6	38.7	-
9.	BrSG.2	38.1	-	26.	MP30.1	48.7	+
10.	BrSG.3	65.4	-	27.	BCK10.2	35.0	+
11.	BrSG.5	50.0	-	28.	BCK15.4	41.9	-
12.	BrSG.7	43.1	-	29.	BCK15.7	35.4	-
13.	Bp25.1	47.6	-	30.	BWK50.1	50.9	+
14.	Bp25.2	59.1	-	31.	BCC15.2	32.0	-
15.	Bp25.3	47.9	-	32.	BCC15.11	35.0	-
16.	Bp25.4	43.5	-	33.	BTC20.4	29.9	-
17.	Bp25.5	49.4	-	34.	BTC20.6	44.1	+

Table 7.15. Moler disease incidence of shallot treated with rhizobacterial strain with dipping and drenching methods in the glasshouse trial

Bacterial strains	Disease incidence (%)	
	Dipping method	Drenching method
BrSG.3	0 a	0.0 a
BrSG.5	6.7 a	0.0 a
BrSM.4	13.3 a	20.0 a
Bp25.2	13.3 a	6.7 a
Bp25.6	0.0 a	0.0 a
Bp25.7	0.0 a	0.0 a
Kontrol (+)	100.0 b	100.0 b
Kontrol (-)	86.7 b	73.3 b

7.2.4 Tissue culture for virus removal in alliums

Indonesian tissue culture research aimed at eliminating viruses in infected Shallot bulbs.

Research was conducted aimed at eliminating potyvirus and carlavirus from shallot bulbs using a combination of thermotherapy (30°C, 37°C, in homogenous and heterogenous condition) and chemotherapy (ribavirin 10 mg L^{-1} and 25 mg L^{-1}) with different size of explant shoot tip culture (1 mm, 2 mm, 3 mm). The results from the study that evaluated the effects of chemotherapy, thermotherapy and explant size showed that plantlets derived from 1 mm-explant were virus-free and the treatment combinations of 2 mm-explants in heterogenous condition reduced virus incidence by 50–67%. A treatment combination of thermotherapy and chemotherapy alone did not produce virus-free plantlets.

Heat treatment is a common method used to produce virus-free plants (i.e. using hot water or hot vapour treatment). Hot water treatment is generally applied to dormant organs, such as seeds or bulbs. This research aimed to develop virus-free shallot plantlets through hot water treatment combined with tissue culture techniques. The experiment was done using a completely randomized design with three factors, including cultivars ('Sumenep' and 'Bima Curut'), temperature (45°C, 50°C, and room temperature), and soaking time (15 and 30 min). Detection of the virus using dot immuno binding assay before treatment confirmed the infection of *Onion yellow dwarf virus* (OYDV), *Shallot latent virus* (SLV), and *Garlic common latent virus* (GCLV) in bulbs. In general, hot water treatment in combination with tissue culture did not affect the ability of plantlets to grow. Neither potyvirus nor carlavirus were detected in plantlets of cv. 'Bima Curut' derived from bulbs given a hot water treatment at 45°C for 15 minutes. Carlavirus, but not potyvirus, was detected from plantlets cv. 'Sumenep' derived from bulbs given hot water treatment at 50°C for 15 minutes.

Garlic is also an important horticultural commodity in Indonesia. Farmers grow garlic using vegetative bulbs giving increased risk of disease infestation since several diseases are reported on garlic bulbs. Virus infection with a range of viruses is identified including, carlaviruses (*Shallot latent virus* (SLV) and *Garlic common latent virus* (GCLV)), potyviruses (*Onion yellow dwarf virus* (OYDV), *Shallot yellow stripe virus* (SYSV) and *Leek yellow stripe virus* (LYSV)) and the allexiviruses (Arya *et al.* 2006; Chen *et al.* 2004; Dovas *et al*. 2001; Shahraeen *et al*. 2008). Infection with SLV, LYSV, GCLV, and OYDV in Mexico affected garlic quality and yield (Moreno *et al*. 2014). According to Walkey (1990), infection with OYDV and LYSV caused yield losses of 63% and 54%, respectively. Moreover, Bagi *et al*. (2012) reported that OYDV caused a bulb weight reduction of up to 21.5%. Recently, Kadwati and Hidayat (2015) reported that garlic samples from Bogor were infected by GCLV, and SLV. A key preventative strategy in managing garlic viruses is using virus-free planting material. Virus-free garlic plant materials could be established through many approaches, such as apex culture, meristem tip culture, thermotherapy, electrotherapy, chemotherapy, and cryogenic methods (Almaarri *et al*. 2012). Soliman *et al*. (2012) successfully eliminated OYDV from garlic bulbs by an electrotherapy method at 15 mA for 10 min with minimal deleterious effect on plant growth. Similarly, Pangestuti (2013) used electrotherapy at 5 mA for 10 min on garlic bulbs and successfully eliminated up to 28.6% virus infection. The use of thermotherapy at 38ºC for 3 weeks has also been reported to eliminate OYDV from garlic (Soliman *et al.* 2012).

Research evaluated virus elimination methods in garlic bulbs by tissue culture using combinations of electrotherapy (0mA, 5mA, 10mA, 15mA, and 20mA) each at 10 minute duration and thermotherapy (25ºC, 35ºC, 37ºC, and 39ºC) in two garlic cultivars, (cvs. Sangga Sembalun and Lumbu Hijau). The treated plantlets were incubated for four weeks and observations conducted for plant growth characteristics, i.e. number of plantlets survived, number of leaves, and height of plantlets. The research on the effects of electrotherapy (0mA, 5mA, 10mA, 15mA, and 20mA) and thermotherapy (25ºC, 35ºC, 37ºC, and 39ºC) on garlic

virus incidence showed that both thermotherapy and electrotherapy gave similar effect on plantlets growth for cvs Sangga Sembalun and Lumbu Hijau. The frequency of virus infection after treatments was detected from plantlets by reverse transcription-polymerase chain reaction (RT-PCR) using OYDV specific primer and showed that thermotherapy at 33^{o}C was able to eliminate virus infection.

Australian research on garlic tissue culture and virus elimination

Queensland has a small but developing garlic industry primarily located in the Lockyer Valley. The major constraints to local production has been identified as viruses and a range of other diseases resulting in low and unreliable yield, small bulbs and quality issues. The main aim of this tissue culture activity was to identify the suite of viruses in Australian garlic and to identify or develop virus free plants. Despite extensive screening, no virus free garlic plants were found. Tissue culture is used to eliminate viruses in vegetative plant material, however, research is needed to develop virus elimination methods for Queensland garlic since tissue culture alone will not eliminate the virus, which multiply rapidly in in vitro plants.

Effect of temperature

Research was conducted to examine the effect of temperature for suppression of virus and for effects on garlic germination. In a preliminary storage germination trial, high temperature was shown to suppress virus symptoms and thermotherapy was used in the tissue culture program as one of the virus elimination strategies. We investigated methods of culture establishment, including shoot-tip culture, basal plate culture, meristem culture and use of bulbils produced in the inflorescence as source explants. There have been problems with garlic tissue culture, with cultures failing to grow and multiply possibly due to the presence of the virus. No virus free garlic plants were identified from field selections so all starting material was infected with a suite of many viruses.

High temperature applied during garlic bulb storage was investigated to determine if this reduced virus titre. The temperature was increased gradually to bulbs stepping up in weekly exposure to 30^{o}C, 33^{o}C, 35^{o}C and 37^{o}C, at which the temperature of the bulbs were held for four weeks. Cloves from several bulbs were mixed together. A selection of 36 large cloves and eight (8) small cloves were each used in a heat treatment verses control treatment where cloves were stored at room temperature. Cloves were planted into 50:50 peat sand steam pasteurized potting mix. The heat treatment of cloves delayed germination by 2 weeks. At eight weeks after planting, three of the 44 heat treated cloves had failed to germinate, while in the room temperature treatment (control) all cloves germinated. However, 19 of the control plants subsequently died whilst none of the heat treated plants died. The plants generated from heat treated cloves were vigorous with upright broad dark green leaves but there was some flecking and light streaking symptom (Fig. 7.6). The plants grown from control cloves had poor vigor, curled twisted leaves with mosaic, flecking, streaking and a wide yellow band along leaves. It is likely heat treatment eliminated or suppressed some of the viruses, particularly those that more severely affected growth.

In a second experiment low temperature was applied during storage to evaluate effect on germination. Garlic cloves were stored under five different temperatures including RT (Room temperature - average 28^{o}C for 8 weeks), COLD (4^{o}C for 8 weeks), HEAT (High temperature - gradually increased to 37^{o}C and held for 5 weeks), HEAT then COLD (high temp, then 4^{o}C for 5 weeks) and HEAT then RT.

Heat treated Room Temperature

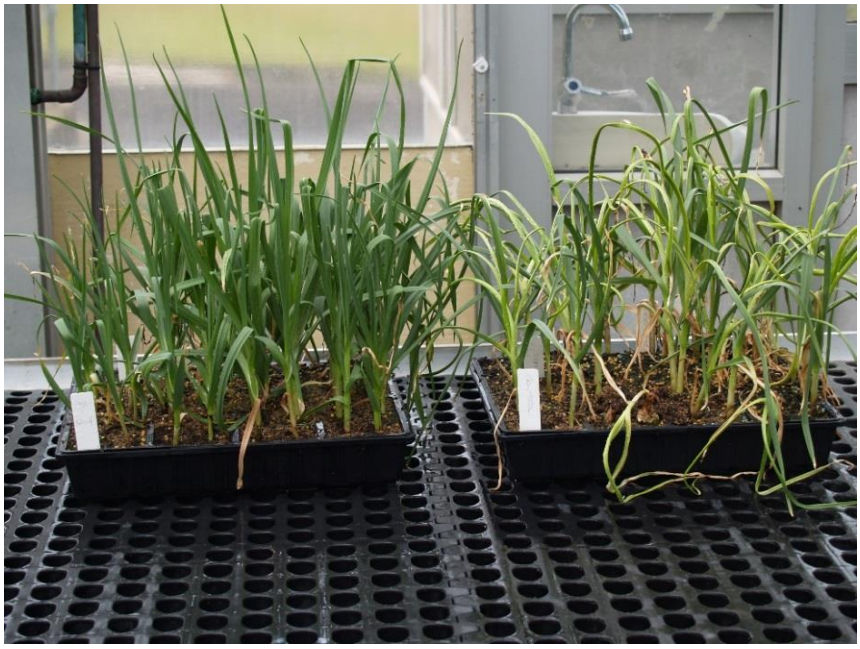


Figure 7.6. Effect of heat treatment of garlic cloves on the growth and health of garlic plants.

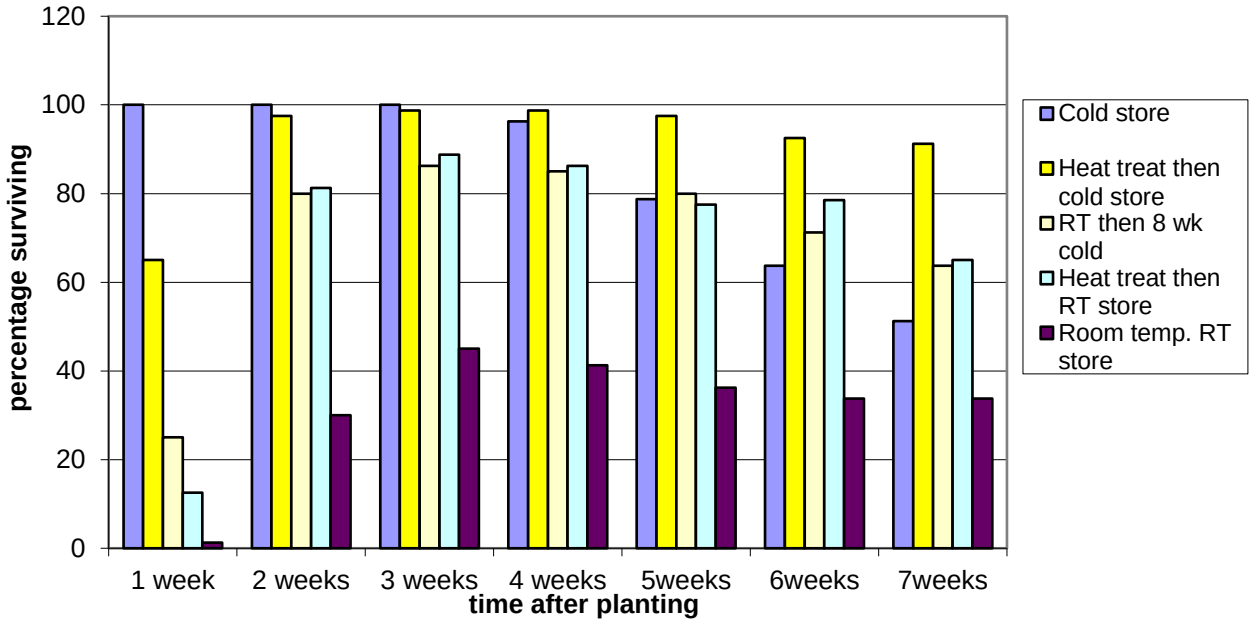


Figure 7.7 Garlic plants surviving after seed storage under different heat treatments.

Cloves were planted into 50:50 peat sand steam pasteurized potting mix. For the COLD treatment 98% of the cloves germinated within 5 days of planting. In the remaining treatments germination commenced at one week after planting (WAP) by which stage 100% of cold treated cloves had germinated. The next best germination response was from HEAT, then COLD where 65% had germinated by 1 WAP and by 2 WAP 98% had germinated. The RT

then Cold and Heat then RT treatments both reached 85% germination by 3 WAP and the RT treatment had only 43% germination by 21 days. After 3 WAP all treatments declined and there was some plant death (Fig. 7.7 and 7.8). At 7 WAP the best treatment was Heat then cold with 90% of plants growing. In all other treatments less than 70% were alive by 7 WAP and only 35% alive in the room temperature treatment. The cold treatment while it induced good germination also had high disease levels with only 50% surviving at 7 WAP.

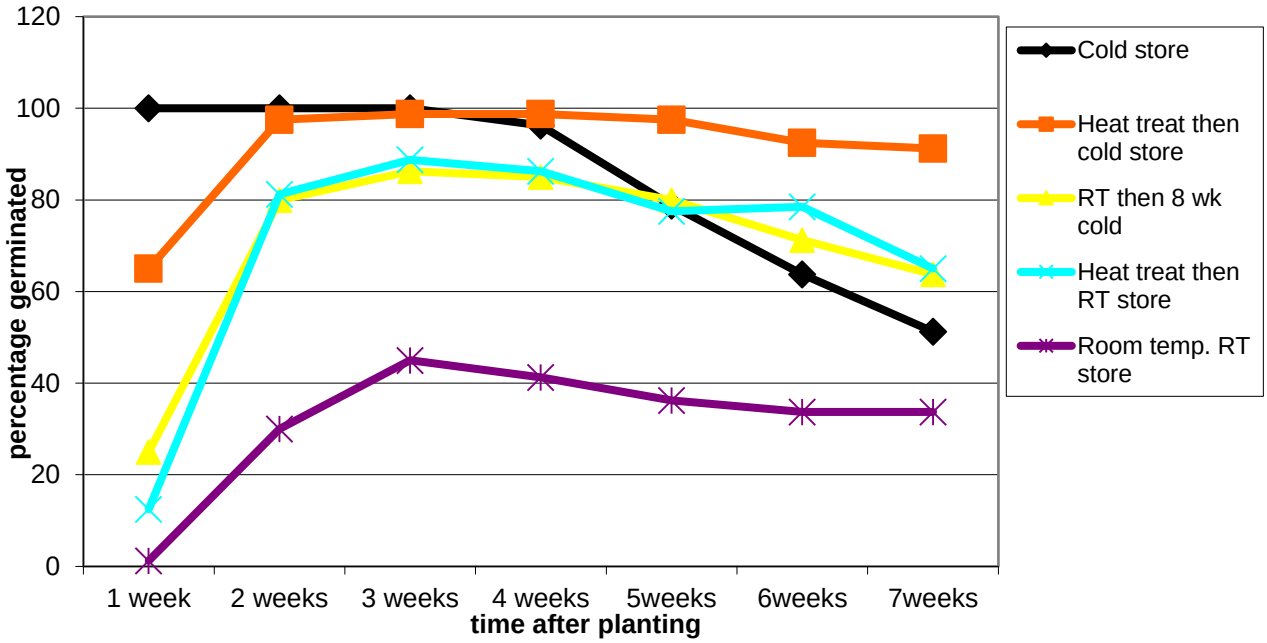


Figure 7.8 Percentage garlic plants emerging after various seed storage treatments.

Effect of Temperature and clove size on germination

Cloves stored at room temperature for seven months after harvest were used for initiation by shoot-tip culture and basal plate culture into MS media containing 2.5mg/L Benzyl amino purine (BAP) solidified with 2.5g/L phytagel and pH adjusted to 6.0 at 26^{o}C under 12 hour light. Shoot-tip contains meristem and small amount of surrounding tissue. Basal stem disc was a 2 mm slice from base of cloves that contained root and meristem and axillary bud initials. No differentiated growth was seen in any treatment and no cultures were established.

It was hypothesized that this may have been due to age and desiccation of cloves or lack of cold pre-treatment. In 2013 experiments looked at initiation treatments (shoot-tip or basal plate culture) that included storage at room temperature, heat treatment with or without cold storage alone or cold pre-treatment. All cold treatments improved germination in both shoot-tip and basal plate culture (Fig.7.9). The room temperature treatments were the poorest with only 15% of basal plate cultures producing shoots compared with 85% in the cold store treatment. Basal plate cultures initiated from cloves exposed to heat treatment followed by cold pre-treatment had 89% with shoots.

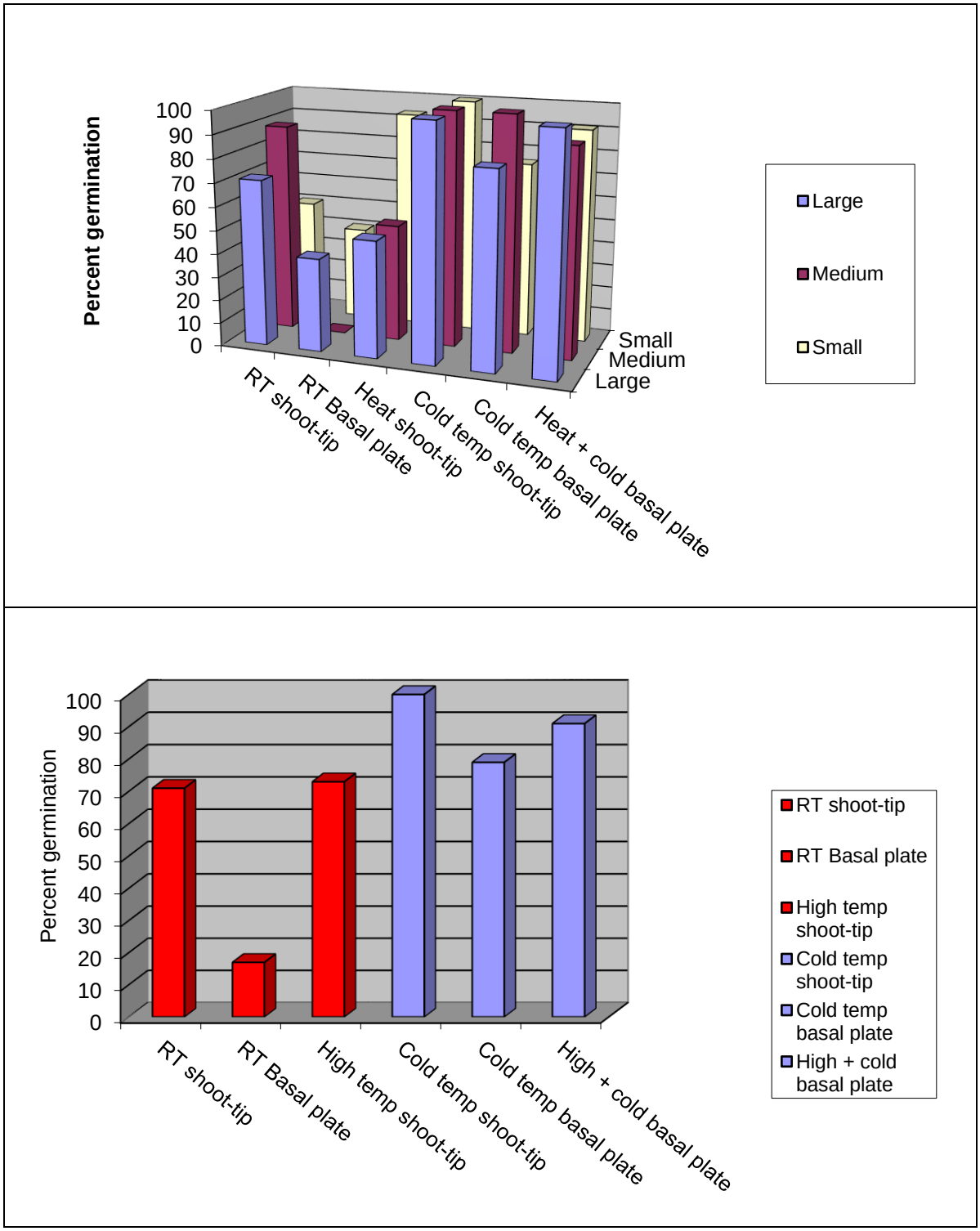


Figure 7.9. Effect of temperature and clove size on germination of garlic.

Bulbils were initiated into culture since it has been reported that they may have lower levels of virus. These bulbils were very slow to germinate and remained in culture for a long time as very small fine garlic plants. The explants rarely multiplied by producing shoots but instead produced callus. Further research is required to optimise tissue culture protocols for Queensland tropical garlic cultivars.

Virus elimination

Well established and multiplying garlic explants were used in research experiments aimed at elimination of viruses. Twenty-five individual explants were selected per treatment and each was put into a 125ml container containing 25 mls of medium. Explants were exposed to treatments over two successive subculture cycles 6 weeks apart and were cultured at 22-24^{O}C under 12 hr light. Two treatments were applied, one using antiviral agent Ribavirin in tissue culture media and the other a tissue culture control. It was important to include the tissue culture control because in some cases virus can be eliminated during tissue culture production of plantlets. The two treatments were 25mg/L Ribavirin (filter sterilised) added to sterilised MS with 3% sucrose 0.1mg/L NAA + 1mg/L BAP solidified with 2.0 g/L phytagel, and control with same medium excluding Ribavirin .

After the two treatment applications survival was recorded. There was no significant difference between treatments, with 16 from 25 explants surviving the ribavirin treatment and 14 from 25 in the control. After a further four subculture cycles, multiplication and survival was recorded. The Ribavirin treated plants had higher survival with 14 of the 16 still alive compared with the control where only 7 of the 14 were still alive. However, multiplication was similar for both treatments with the Ribavirin treated explants producing an average total multiplication of 19 clumps per plant compared with the control explants (17 clumps per plant). However, for both treatments the multiplication was not uniform for each explant, with a multiplication factor ranging from 1 to 45 times. The population of explant in the experiment were multiplied until a minimum of ten plants was produced from most explants.

The explants were multiplied so that a subset of plants from each treatment could be grown on in a controlled plant growth cabinet and tested for virus after they had grown for a minimum for 3 months or longer while the other plants derived from each explant were maintained and continued multiplication in vitro. The strategy being that if virus free or virus reduced plants were produced the line producing the plants could be available in tissue culture to be used for further screening for production of more virus free plants. There was large variation in explant size at deflasking as well as the rate of plant growth. There was also variation in the amount of bulb development and rooting on individual explants within a culture vessel. The plantlets with well-developed bulbs and roots survived acclimatisation, however, explants with little bulb development and few roots did not survive. Those plants that survived in the first three weeks after acclimatisation continued to grow well. Thirty randomly selected plants from lines across both treatments were grown in the preliminary trial to determine if any plants were free from virus or had reduced virus. After 3 months some of the plants began to show symptoms of virus with chlorotic streaks and mosaic and twisted growth of leaves, most of these plants were from lines in the control treatment (tissue culture without chemotherapy) (Fig. 7.10). The remaining plants were symptomless after 3 months growth, however, there was variable growth responses in the plants and many of the symptomless plants were much smaller than the other plants.

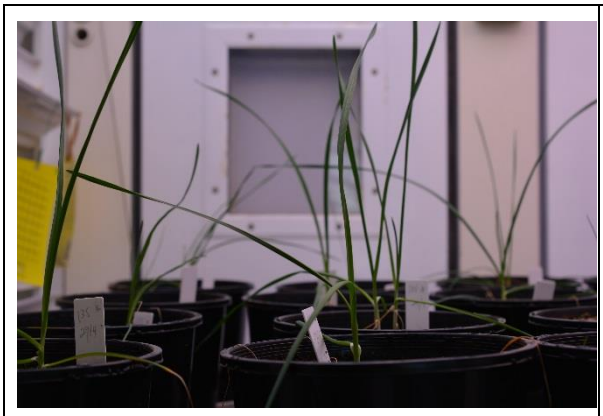
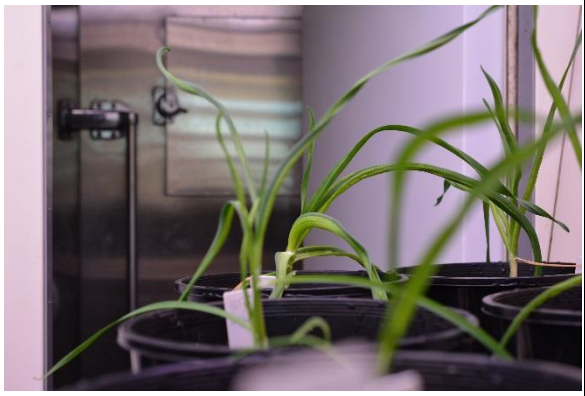
	
With chemotherapy	No chemotherapy

Figure 7.10 Effects of chemotherapy on tissue cultured garlic plants showing virus symptoms in untreated control plants.

Leaf samples from 30 plants that had been grown for a minimum of three months were collected for virus diagnostic assay. The leaf samples were collected and total nucleic acid extracted. There was a high level of success in eliminating Allexi, Carla and Poty virus from garlic using a combination of tissue culture with chemotherapy (Ribavirin) (Table 7.16). All control treatments tested positive to Alexi virus. To determine if all plants in a treated line produced a consistent result 13 plants from line 11 were included in the first evaluation. All plants were free from virus. One of the TC & Ribavirin lines failed to remove Allexi and Carla virus and all three plants from that line contained virus (refer to Table 7.16).

Further research should be undertaken using virus free plants and populations from selected lines should be multiplied, induced to elongate and develop bulbs and roots prior to acclimatisation. Tissue culture derived plants need to be grown in a glasshouse in suitable cool environment and samples taken to confirm virus freedom. It will take one season to produce the small cloves and the second season to understand how the small cloves compare to yield of conventional cloves. A population of plants would then be used for a field trial to compare with control (tissue culture derived with virus) and also conventional garlic to understand the real impact on yield. If positive effects on yield and quality can be obtained using virus reduced or virus free plants then the program would need to expand to a wider range of cultivars. Improvements in the tissue culture production will also be required.

Prior to this project there was no Allium tissue culture program in Queensland and a significant outcome of the project has been the development of research capacity in allium tissue culture propagation in Australia and Indonesia. This project has provided the means to build Allium tissue culture capacity necessary to undertake research to develop clean plants and eventually to establish a clean plant scheme. Work is ongoing to develop a reliable micropropagation system since multiplying cultures are required for any further work in virus elimination. Tissue cultured plantlets were grown on in the nursery for several months before virus indexing. The project has not established virus free garlic planting material, however, the important first steps have been taken to develop skills and capacity in Australia and Indonesia to position partners in being able to produce virus free garlic plants necessary in undertaking trials to understand impact from virus/s on garlic crop productivity and to understand vector transmission and time to reinfection. These research components are needed in order to identify the need for a clean garlic seed bulb supply system. Further research is required to improve the garlic tissue culture production that will be viable at a commercial level.

Table 7.16. Garlic Virus Diagnostics- Virus Free Tissue Culture

	RT-PCR				
Sample	**Allexi**	**Carla**	**Poty**	**Treat**	**TC CODE**
1	Negative	Negative	Negative	TC & Ribavirin	11
2	Negative	Negative	Negative	TC & Ribavirin	11
3	Negative	Negative	Negative	TC & Ribavirin	11
4	Negative	Negative	Negative	TC & Ribavirin	11
5	Negative	Negative	Negative	TC & Ribavirin	11
6	Negative	Negative	Negative	TC & Ribavirin	11
7	Negative	Negative	Negative	TC & Ribavirin	11
8	Negative	Negative	Negative	TC & Ribavirin	11
9	Negative	Negative	Negative	TC & Ribavirin	11
10	Negative	Negative	Negative	TC & Ribavirin	11
11	Negative	Negative	Negative	TC & Ribavirin	11
12	Negative	Negative	Negative	TC & Ribavirin	12
13	Negative	Negative	Negative	TC & Ribavirin	13
14	Negative	Negative	Negative	TC & Ribavirin	14
15	Negative	Negative	Negative	TC & Ribavirin	11
16	Negative	Negative	Negative	TC & Ribavirin	11
17	Negative	Negative	Negative	TC & Ribavirin	7
18	Positive	Negative	Negative	TC & Ribavirin	9
19	Positive	Positive	Negative	TC & Ribavirin	9
20	Positive	Negative	Negative	TC & Ribavirin	9
21	Negative	Negative	Negative	TC & Ribavirin	15
22	Negative	Negative	Negative	TC & Ribavirin	15
23	Positive	Negative	Negative	TC Control	33
24	Positive	Negative	Negative	TC Control	33
25	Positive	Negative	Negative	TC Control	33
26	Negative	Negative	Negative	TC & Ribavirin	6
27	Negative	Negative	Negative	TC & Ribavirin	6
28	Positive	Negative	Negative	TC Control	32
29	Positive	Negative	Negative	TC Control	32
30	Positive	Negative	Negative	TC Control	32

7.2.5 Integrated crop management strategies for managing pepper yellow leaf curl virus in chilli.

Screening germplasm for disease resistance.

Chili germplasm used in the screening activities came from different sources, including commercial cultivars available in Indonesia (7 cultivars), AVRDC accessions (47 lines), IPB collection (17 lines), and DAF's collection (40 lines). Seed of AVRDC accessions were provided by IVEGRI, and included chili lines from the previous ACIAR project (HORT/2004/048) while seed of Queensland DAF collections were received from USDA.

After inoculation most of the chili genotypes developed PYLCV symptoms, although the incubation period and symptom expression varied from plant to plant. The shortest incubation period was five days after inoculation (DAI), whereas the longest was 29 DAI (Tables 7.17, 7.18, 7.19 and 7.20). Unique symptoms of PYLCV infection were observed, including (1) dark green and yellow mosaic, (2) yellow mosaic and reduction of size leaves, (3) yellow mosaic and leaf cupping upward, (4) yellow mosaic and leaf cupping downward and (5) rugose (Fig 7.11).

An important challenge when working with different chili pepper genotypes is the variability in symptom expression. Difficulties in the determination of visual scoring for resistance influences the conclusion regarding the response of the chili pepper genotypes to PYLCV. In our screening study, there were some lines that could be considered as having resistance to PYLCV based on number of plants with disease and the type of symptom. IPB lines CH3, C12, C19, and UNIBCG are potential sources of resistance to PYLCV; having 0% disease incidence (Table 7.19). The evaluation of these particular lines should be repeated several times to confirm the response. In a repeated evaluation for IPBCH12 the resistance response was different from one test to another (Table 7.19). The variable result might be due to different seed lots and segregation that may have occurred during seed propagation.

In addition to screening the germplasm collection, crossing experiments using Warlock and IPB C 12 as parents were done and seed of the F2 and F3 generation were obtained (F2012328 and F3012328 respectively) and has screened for PYLCV resistance (Table 7.19). Breeding for PYLCV resistance should continue, since the farmers in Indonesia need resistant cultivars to obtain high yields. More germplasm might need to be screened to be used as resistant parents for the breeding program. In India, Kumar *et al.* (2006) screened 307 chilli and sweet pepper genotypes belonging to *Capsicum* species against PYLCV, and only three genotypes (*viz.* GKC-29, BS-35 and EC-497636) showed no symptom development under field conditions. Most chili genotypes were susceptible to PYLCV.

Table 7.17. Evaluation of resistance to PYLCV in commercial Indonesian chilli cultivars.

Lines	No. plants	No. plants showing symptom	Disease incidence (%)	Incubation period (days after inoculation)
Bara	15	11	73.3	10-25
Biola	15	9	60.0	11-22
Carisa	15	14	93.3	9-29
Impalo	15	9	60.0	9-26
Laris	15	8	53.3	15-24
Luwes	15	10	66.7	9-17
Surya	15	7	46.7	8-20

Table 7.18 Evaluation of resistance to PYLCV in chilli germplasm sourced from the AVRDC collection.

Lines	No. plants	No. plants showing	Disease incidence	Incubation period (days after

		symptom	(%)	inoculation)
0735-5617-1	15	4	26.7	14-27
0735-5623-1	15	7	46.7	10-23
0735-5646-1	15	7	46.7	7-18
0735-5649-1	15	8	53.3	9-16
0735-7641-B	15	11	73.3	7-15
0735-5636-1	15	12	80.0	7-29
0735-5670-1	15	15	100.0	7-19
0735 - 5677	15	15	100.0	7-15
0735 - 5696 - 1	15	15	100.0	7-15
0735 - 5604 - 1	15	15	100.0	7-15
0735 - 5611 - 1	15	15	100.0	7-15
0735 - 5617 - 1	15	15	100.0	7-15
0735 – 5623 - 1	15	15	100.0	7-15
0735 - 5626 - 1	15	15	100.0	7-15
0735 - 5629 - 1	15	15	100.0	7-15
0735 - 5649 - 1	15	15	100.0	7-15
0737 - 7732 - B	15	15	100.0	7-15
PP 0537 – 7558 - 1	15	15	100.0	7-15
A1 Lorai	15	15	100.0	7-15
A18 CCA 7433 - 4 -2	15	15	100.0	7-15
A22 CCA 7446 - 1 - 6	15	15	100.0	7-15
A29 CCA 7452 -2-4	15	15	100.0	7-15
AUPP 0719	3	2	66.7	7-19
AUPP 1004-B	5	5	100.0	7-19
AUPP 0906	3	2	66.7	7-19
AUPP 0207	1	1	100.0	11
AUPP 0717	4	3	75.0	7-19
AUPP 0712	4	2	50.0	7-19
AUPP 0718	7	2	28.6	7-19
AUPP 1102-B	6	4	66.7	7-19
AUPP 1003-B	7	5	71.4	7-19
AUPP 0514	9	4	44.4	7-19
AUPP 1002	3	2	66.7	7-19
AUPP 1103-B	3	3	100.0	7-19
AUPP 1104	1	1	100.0	12
AUPP 0713	5	4	80.0	7-19
AUPP 0513	5	5	100.0	7-19
AUPP 0708	3	2	66.7	7-19
AUPP 0716	3	1	33.3	11
AUPP 0704	4	4	100.0	7-19
AUPP 9813	4	4	100.0	7-19
AUPP 0715	6	1	16.7	13
AUPP 0805	9	6	66.7	7-19
AUPP 0205	7	7	100.0	7-19
VICTOR	2	2	100.0	7-19
KAISER	9	7	77.8	7-19
C-LANTING LBG-1	3	3	100.0	7-19

Table 7.19. Evaluation of resistance to PYLCV in chilli germplasm sourced from the IPB collection.

Lines	No. plants	No. plants showing symptom	Disease incidence (%)	Incubation period (days after inoculation)
IPB C12 (2nd test)	15	7	46.7	11
IPB C12 (3rd test)	15	6	40.0	7 - 13
IPB C12 (4th test)	32	27	84.4	5 - 19
IPB C12 UNIB	29	4	13.8	23 - 25
IPB C5	22	11	50.0	14
F2012328 (1st test)	19	13	68.4	4 - 21
F2012328 (2nd)	17	9	52.9	5 -11
F3012328 2	9	7	77.8	7 - 12
CH3	28	0	0.0	-
IPB C12 (1st test)	30	0	0.0	7 - 12
Y uni	30	2	6.7	7 - 12
Ames	25	1	4.0	11
IPB (1 x 3)	30	11	36.7	7 - 13
IPB (5 x 3)	30	14	46.7	7 - 13
IPB (6 x 5)	30	12	40.0	7 - 13
IPB (6 x 3)	30	9	30.0	7 - 13
IPB C19	29	0	0.0	-
IPB C8	29	4	13.8	7 - 13
Kopay	25	0	0.0	-
UniBCG	20	1	5.0	10
IPB C10	30	23	76.7	7 - 13
IPB C14	30	10	33.3	7 - 13

The presence of genes for resistance will allow the development of an F2 population which can be screened and phenotyped. The subsequent identification of a marker for resistance genes will allow for more effective and efficient screening for resistance and easier incorporation of resistance into commercial lines. A field survey of viruses in chilli crops was conducted and the results from this survey directly impacts on the development of begomovirus resistant germplasm in Indonesia and a formal identification of the suite of viruses affecting solanaceous crops in Indonesia. The development of resistant germplasm needs to be underpinned by accurate diagnostics on the virus species present and the detection of new begomovirus species may necessitate further screening of germplasm to identify new resistance genes. The publishing of this data will allow for further research across Asia to develop strategies to better manage viruses in chillis and peppers.

Table 7.20. Evaluation of resistance to PYLCV in chilli germplasm sourced from the Queensland DAF collection (USDA lines).

DAF Lines	No. plants	No. plants showing symptom	Disease incidence (%)	Incubation period

				(days after inoculation)
69-3 1st	16	2	12.5	58
69-3 2nd	20	16	80.0	11 - 20
Warlock 1st	34	4	11.8	11
441-642	22	13	59.1	6
Warlock 2nd	21	16	76.2	7 - 22
71-3-1	11	4	36.4	5
2-1-SPS	11	6	54.5	7
40-5	22	7	31.8	20
B-13	19	12	63.2	10
B-29	15	7	46.7	24
B63 RED	30	18	60.0	9
D2 PLANT a 159 282	17	9	52.9	8
B 27	21	13	61.9	7
P1 224706	16	12	75.0	6
B50 TYPE RED 2013	30	30	100.0	7
B44 RED 2013	29	29	100.0	7
B1	30	30	100.0	6
PI 238 057	17	17	100.0	9
42-3	30	30	100.0	8
197000	15	9	60.0	5 - 19
181907	15	12	80.0	8 - 21
B46 TYPE A 2013	22	10	45.5	6 -21
B64 2013	15	11	73.3	15 -26
B48	14	9	64.3	4 -22
B58A 2014	30	22	73.3	8 - 15
45-1	18	10	55.6	8 - 14
B41 2014	30	11	36.7	10 - 21
B52A 2014	29	10	34.5	11 - 23
257045	17	15	88.2	15 - 24
B722014	7	5	71.4	15 - 24
B51 203	11	9	81.8	15 - 24
B67	16	10	62.5	4 -16
24-4	7	6	85.7	14
B5 2013	25	10	40.0	8 - 14
B49B	31	24	77.4	9 - 15
B37B	30	13	43.3	11 - 21

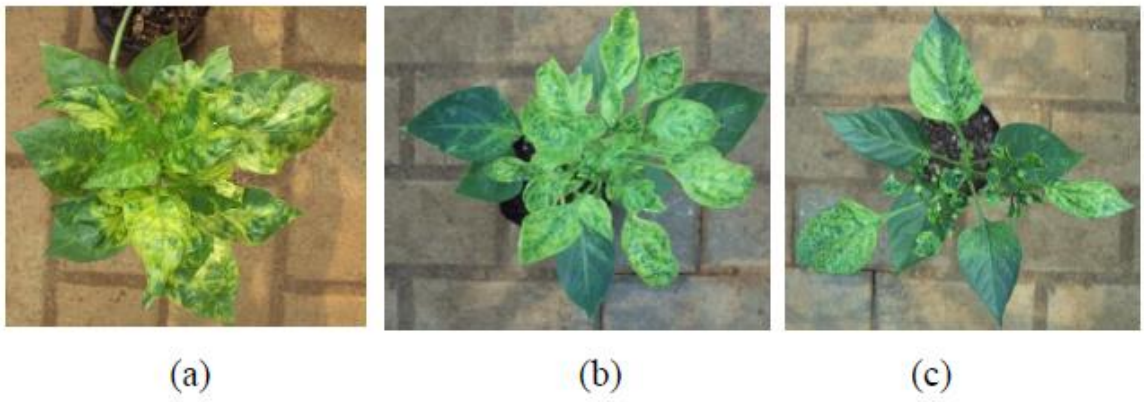


Figure 7.11. Symptoms types of PepYLCV infection on chili germplasms : yellow mosaic (a); green and yellow mosaic (b); small leaf and cupping (c).

An experiment evaluated the effects of border cropping and mineral oil application on PYLCV incidence. Disease symptoms started to develop one month after chili plants were transplanted to the field. Observations on disease incidence indicated that border crops inhibited virus infection (Fig 7.12). Furthermore, corn was a better border crop option than yard long bean. Application of mycorrhiza tended to reduce disease incidence but the mineral oil and commercial insecticide had variable activity.

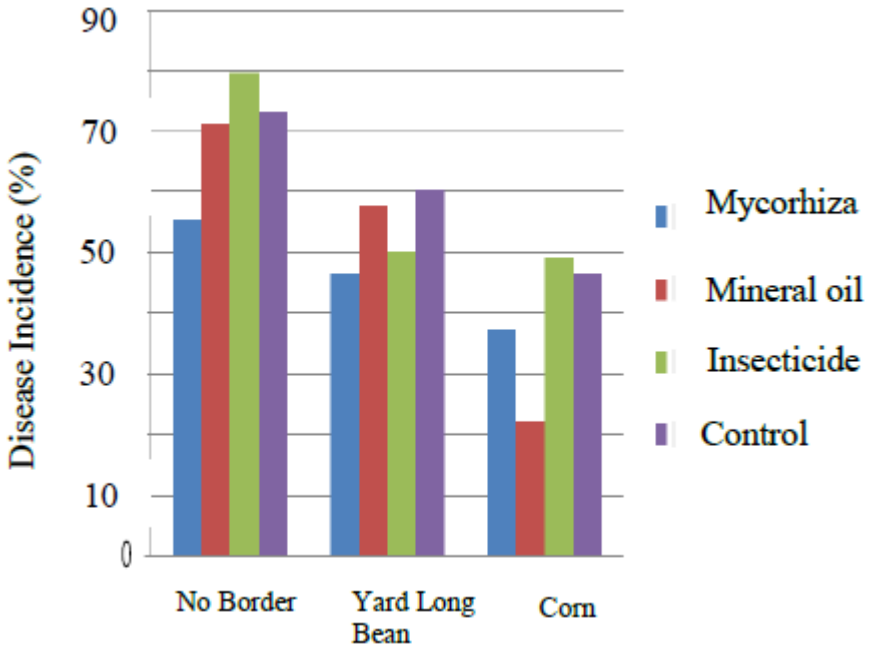


Figure 7.12. The effect of border crops in combination with mineral oil, commercial insecticides and mycorrhiza on incidence of yellow leaf curl disease in chili pepper

7.3 Nutrient management and agronomy in shallot and garlic

7.3.1 Nitrogen management in Indonesian shallots

Partial nutrient budgeting in shallots

The data collected in the partial nutrient budget included mean data for yield components of plant population as well as fresh and dry matter yield for foliage and bulbs (Table 7.21). Plant populations varied considerably across districts and within districts (farmer to farmer). The lowest plant population was recorded at Brebes at 290,000 plants ha^{-1} and the highest population was almost twice this (560,000 plants ha^{-1}) at Cirebon. The foliage fresh yield also varied considerably across sites ranging from 2.1 to 33.9 t ha^{-1}. The variability in crop foliage yield is an indicator of variability in crop health and vigour observed in shallot production in Indonesia. The mean bulb fresh yield in Cirebon was 26.8 t ha^{-1} and higher than the mean yield recorded for Brebes (18.1 t ha^{-1}), however, bulb fresh yield, dry matter concentration (DM%) and dry matter yield all tended to be much less variable than was the data for foliage samples. These values were also considerably higher than the values included in official data but consistent with findings of Woldetsadik and Workneh (2010) that showed the at-harvest fresh yield of shallot bulbs was about 21.6 t ha^{-1} with a DM% of 16.7% and the cured bulb yield was 18.5 t ha^{-1} with a DM% of 18.1%. The bulb DM% in this study had relatively low variability ranging from 13.8% to 17.6%.

Over application of N, P, K and S fertilizer was evident at both Cirebon and Brebes where generally the amount of nutrient applied was greater than whole crop requirement (Table 7.21). On average, application of N at both sites was about twice that of whole crop requirement and for P application was about five times whole crop requirement. The farmers surveyed in the Brebes region tended to have better balanced nutrient inputs, where nutrient application tended to more closely meet crop requirement. Indeed, several farmers at Brebes applied fertilizer at rates slightly less than whole crop requirement. It is possible that shortfalls in nutrient application by individual farmers is met by losses from other high use farmers through water movement in the complex linkage of irrigation/water channels across regions under the Surjan system of farming. The fate of the excess applied fertilizer was not evaluated in this study but consistent evidence of algal blooming in Surjan channels supports the notion some N is lost to Surjan channels. Fertilizer represents approximately 30% of farmer crop input costs and improvements in fertilizer application and management would provide substantial financial and environmental benefits. The issue of the fate of fertilizer losses and the dynamics of N movement in shallot chilli systems requires focussed research attention.

Macronutrient concentrations including means, standard errors and range of values for shallot bulb and foliage is presented in Table 7.22. For each of these nutrients, with the exception of P, the values are at about the expected sufficiency levels. The P concentration in foliage however varied from values that were marginal to low. Seven of the 12 samples had P concentrations under 0.2%, which would be considered marginal. This is despite the fact that most farmers applied P at rates about five times that of removal. In the present study the mean bulb tissue S concentrations were at or above an S sufficiency concentration of 0.27% (Coolong *et al.*, 2004). The lowest bulb S concentration was recorded at Cirebon (0.21%) and the Brebes samples tended to have much higher S concentrations (mean of 0.44%) than Cirebon samples (mean of 0.27%) suggesting that S status of shallot crops could be important.

Table 7.21. Data for major element (N, P, K, and S) uptake and partitioning (kg ha^{-1}) between foliage and bulbs and applied fertiliser (kg ha^{-1}) collected in a survey of farmers shallot crops in Brebes and Cirebon, Java, Indonesia in 2014.

	Cirebon				Brebes			
	Foliage	Bulb	Total	Nutrient Applied	Foliage	Bulb	Total	Nutrient Applied
Nitrogen (kg ha^{-1})								
mean	31.6	66.0	97.6	198	18.4	61.7	80.1	152
SE	11.0	8.7	15.5	25.3	3.1	6.5	9.5	55.0
range	13.8-67.9	44.7-92.0	58.5-143	140-277	7.5-26.5	42.7-82.9	50.2-109.	59-354
Phosphorus (kg ha^{-1})								
mean	2.8	10.6	13.5	77	1.5	7.6	9.1	49
SE	1.1	1.8	2.4	8.5	0.3	0.9	1.2	26.2
range	1.20-7.02	7.35-15.7	8.55-21.4	50-98	0.56-2.46	5.51-10.3	6.16-12.8	11-148
Potassium (kg ha^{-1})								
mean	34.6	47.9	82.5	149.9	31.3	39.5	70.8	111.6
SE	11.4	6.6	14.6	21.8	6.1	6.3	12.3	42.8
range	15.1-72.1	36.4-67.2	54.3-134.	72-185	15.5-51.2	25.2-61.3	40.7-112.	31-238
Sulfur (kg ha^{-1})								
mean	4.9	11.6	16.5	37.9	4.1	12.4	17.2	42.4
SE	1.7	1.5	2.4	10.3	1.0	1.1	2.6	21.4
range	2.1-10.6	8.6-16.6	10.7-23.9	12-68	1.8-7.4	10.0-16.3	11.8-23.7	10-102

Foliage tissue N concentrations did not directly relate to N application rates where farmers that applied the highest N application rate (up to 355 kg ha^{-1}) had a foliage N concentration (2.11%) that was lower than that of other farmers samples where lower N application rates were applied.

Table 7.22. Nutrient budget survey data for major element (C, N, P, K, Ca, Mg and S) and sodium concentration (%) in dried samples of shallot bulb and leaf tissue collected from Cirebon and Brebes, Java, Indonesia in 2014.

		Nutrient concentration (%)							
		C	N	P	K	Ca	Mg	S	Na
Foliage Samples									
Cirebon	mean	33.3	2.6	0.17	4.0	3.0	0.58	0.42	0.58
	SE	0.54	0.28	0.02	0.40	0.09	0.02	0.08	0.07
	range	31.6-34.6	1.93-3.36	0.11-0.22	3.06-5.40	2.90-3.37	0.53-0.63	0.28-0.69	0.44-0.77
Brebes	mean	37.5	2.3	0.15	4.2	3.6	0.58	0.45	0.54
	SE	2.17	0.24	0.02	0.48	0.20	0.04	0.05	0.06
	range	32.3-44.0	1.73-2.85	0.10-0.20	3.26-5.94	3.17-4.08	0.48-0.68	0.36-0.58	0.36-0.74
Bulb Samples									
Cirebon	mean	34.9	1.36	0.18	1.9	0.68	0.19	0.27	0.09
	SE	0.57	0.07	0.01	0.12	0.05	0.01	0.02	0.01
	range	33.3-36.1	1.14-1.56	0.14-0.20	1.68-2.36	0.57-0.87	0.16-0.22	0.21-0.33	0.06-0.11
Brebes	mean	38.1	2.16	0.25	1.8	0.78	0.18	0.44	0.07
	SE	2.13	0.23	0.01	0.2	0.07	0.02	0.02	0.01
	range	32.4-43.7	1.59-2.88	0.20-0.28	1.54-2.59	0.60-1.00	0.15-0.23	0.37-0.48	0.05-0.08

Foliage and bulb tissue trace element concentrations are presented in Table 7.23 and indicate that, on average, these are all above a sufficient level. Concentrations of both Zn and Cu were high to very high, most likely reflecting the excessive use of Zn and Cu based products for managing plant fungal and bacterial pathogens. The accumulation of these in vegetable soils could be a concern and probably warrants further attention as it potentially poses both an environmental and food safety hazard. The issue of food contamination is more complex since the data for Zn suggests that uptake into the marketable bulb is substantially lower than that in the foliage but for Cu there still appears to be relatively high amounts in the bulb.

On a whole of crop basis, the N, P, K and S budgets for each vegetable crop tended to be positive where nutrient application was far in excess of whole crop uptake. Overall the survey indicates that over application of nutrients is likely to be a serious issue probably impacting on the environment and potentially the marine habitats associated with shallot production. Furthermore, since fertilizer is a substantial cost to farmers there is considerable scope to reduce inputs and improve farmer profitability. The adoption of improved fertilizer management practices provides a positive economic incentive that is likely to deliver good environmental outcomes. If possible the evaluation of soil mineral N at planting and during cropping combined with sap nitrate testing could be useful and relatively inexpensive tools for managing crop N inputs.

Table 7.23. Trace element concentration (Fe, Mn, B, Zn and Cu) (mg kg^{-1} dry matter basis) in dried samples of shallot bulb and leaf tissue collected in a survey of farmers crops from Cirebon and Brebes, Java, Indonesia in 2014.

		Fe	Mn	B	Zn	Cu
Foliage Samples						
Cirebon	mean	3917	365	50	99	22.6
	SE	888	37	10.5	39	6.4
	range	633-5435	279-498	18-74	36-211	10.1-45.5
Brebes	mean	2076	611	73	94	10.2
	SE	325	120	7.33	15	1.3
	range	1468-3298	320-983	57-92	58-128.	7.5-14.8
Bulb Samples						
Cirebon	mean	1820	67	23	26	10.1
	SE	428	5.8	3.2	3.7	1.1
	range	928-3194	48-84	12-30	20-40	8.4-14.3
Brebes	mean	2075	101	31	35	9.5
	SE	516	18.9	3.9	1.7	1.0
	range	784-3274	50-147	23-40	29-38	7.1-13.2

Shallot nitrogen rate trials

This experiment evaluated the effects of increasing N rate on shallot yield. With increasing N application to 320 kg N ha^{-1} field fresh yield, field dried yield, bulb fresh yield and bulb dry matter yield increased (Tables 7.24 and 7.25), but not the bulb DM%. The foliage fresh yield and dry matter yield increased with increasing N rate up to 320 kg ha^{-1}. However, in contrast to the bulb data, the DM% for the foliage was affected by N application rate and decreased from 15.6% in the 0 kg N ha^{-1} treatment to 11.4% in the 320 kg ha^{-1} treatment (Table 7.26). The foliage, bulb and total N uptake increased with increasing N application rate to a maximum of about 70 kg N ha^{-1} total uptake at the highest N application rate of 320 kg N ha^{-1} giving a difference between application and uptake of 250 kg N ha^{-1} (Table 7.27).

Table 7.24. Effect of N application (kg ha^{-1}) on shallot fresh yield (in field) (t ha^{-1}) and dry product yield (field dried) (t ha^{-1}) in a field experiment conducted at Cirebon, Java, Indonesia in 2015.

N rate	Field Fresh Yield (t ha^{-1})		Field Dried Yield (t ha^{-1})	
0	7.7	f	6.2	e
40	13.3	e	10.1	d
80	18.5	d	13.6	c
120	21.3	c	15.4	b
160	21.9	bc	15.8	ab
240	23.5	ab	16.8	a
320	23.9	a	16.9	a
F test Prob.	<.001		<.001	
LSD =	1.69		1.26	

Table 7.25. Effect of N application (kg ha^{-1}) on shallot bulb fresh (BulbFrYld) and dry matter yield (BulbDMYld) (t ha^{-1}) and bulb percentage dry matter concentration (Bulb_DM%) in an experiment conducted in a farmer's field at Cirebon, Java, Indonesia in 2015.

N rate	BulbFrYld (t ha^{-1})		BulbDMYld (t ha^{-1})		Bulb_DM%
0	6.0	f	1.0	e	17.3
40	9.7	e	1.7	d	17.9
80	13.0	d	2.2	c	17.3
120	14.5	c	2.6	b	18.0
160	14.9	bc	2.7	b	17.8
240	16.0	ab	2.8	ab	17.5
320	16.3	a	2.9	a	17.7
F test Prob.	<.001		<.001		0.398
LSD =	1.25		0.20		NS

Table 7.26. Effect of N application (kg ha^{-1}) on shallot foliage fresh and dry matter yield (t ha^{-1}) and foliage percentage dry matter concentration (Fol _DM%) in an experiment conducted in a farmer's field at Cirebon, Java, Indonesia in 2015.

N rate (kg ha^{-1})	FolFrYld (t ha^{-1})	FolDMYld (t ha^{-1})	Fol_DM%
0	1.7 e	0.27 e	15.6 a
40	3.7 d	0.48 d	13.2 b
80	5.6 c	0.66 c	12.0 bc
120	6.9 b	0.78 b	11.4 c
160	7.0 ab	0.81 ab	11.6 c
240	7.5 ab	0.86 ab	11.4 c
320	7.6 a	0.87 a	11.4 c
F test Prob.	<.001	<.001	<.001
LSD =	0.73	0.087	1.23

Table 7.27. Effect of N application (kg ha^{-1}) on shallot nitrogen uptake and partitioning (kg ha^{-1}) between foliage and bulbs and difference between applied fertilizer and N uptake (NAppl-NUptk) (kg ha^{-1}) in an experiment conducted in a farmer's field at Cirebon, Java, Indonesia in 2015.

N rate	Foliage (kg N ha^{-1})	Bulb N (kg N ha^{-1})	Total N (kg N ha^{-1})	NAppl-NUptk
0	4.9 e	11.5 f	16.4 e	-16.4
40	9.2 d	21.3 e	30.5 d	9.5
80	15.0 c	29.6 d	44.6 c	35.4
120	18.4 b	38.7 c	57.1 b	63.0
160	17.9 b	43.5 b	61.3 b	98.7
240	19.3 ab	51.4 a	70.7 a	169.3
320	20.9 a	50.1 a	71.0 a	249.0
F test Prob.	<.001	<.001	<.001	
LSD (5%) =	1.99	2.99	4.52	

A cubit regression was fitted to the fresh yield data with a correlation coefficient (r^2) of 0.99 and identified that the effective maximum yield value was recorded at about 240 kg N ha^{-1} (Fig. 7.13). The fitted functions and response curve for fresh yield and crop N uptake were very similar indicating a direct relationship between crop growth and N uptake (Fig. 7.14). Bulb fresh yield increased significantly from 14.9 t ha^{-1} at an N application rate of 160 kg ha^{-}

^{1} to 16.3 t ha^{-1} at an N application rate of 320 kg ha^{-1}. However, though yield increased with a progressive increase in N application the proportion of N taken up by the crop declined substantially with increasing N application (Fig. 7.15). At the highest yield value of 16.3 t ha^{-1}, recorded at 320 kg N ha^{-1}, the difference between the amount of N applied and that taken up highlighted an excess application over removal of about 250 kg N ha^{-1}, not including an allowance for uptake of mineralized N supplied from the soil. Furthermore, with the significant increase in yield from 160 kg N ha^{-1} to 320 kg ha^{-1} only 9.7 kg of the extra 160 kg ha^{-1} applied N was taken up by the crop. However, this high N application rate is economically justified as the yield increase of 1.4 t ha^{-1} at a modest value of 5,000 rupiah per kg (about AUD $0.50) is valued at 7,000,000 rupiah (AUD $700).

This data highlights that there are massive losses of N from shallot farming systems where high productivity is achieved. This indicates that either the shallot crops are extremely inefficient at N-uptake or management practices associated with shallot cropping result in substantial losses of N. Strategies to improve N use efficiency are required to reduce the cost of fertilizer, improve crop productivity and mitigate environmental losses. This could be achieved through the use of different nitrogen fertiliser formulations and improved splits of application. However, in Indonesia shallot cropping is only 55-60 days from planting to harvest so splitting applications and altered formulations may only provide limited improvement in crop N use efficiency. An investigation into the genetics of N use efficiency and uptake is required to assess the N (nitrate and ammonium) uptake efficiency of current cultivars and to identify whether the genetic basis can be improved. Better knowledge of the loss pathways is required to identify what loss mechanisms are active. The Surjan systems have several potentially major pathways including leaching, overland flow and volatile losses, quite probably as nitrous oxide.

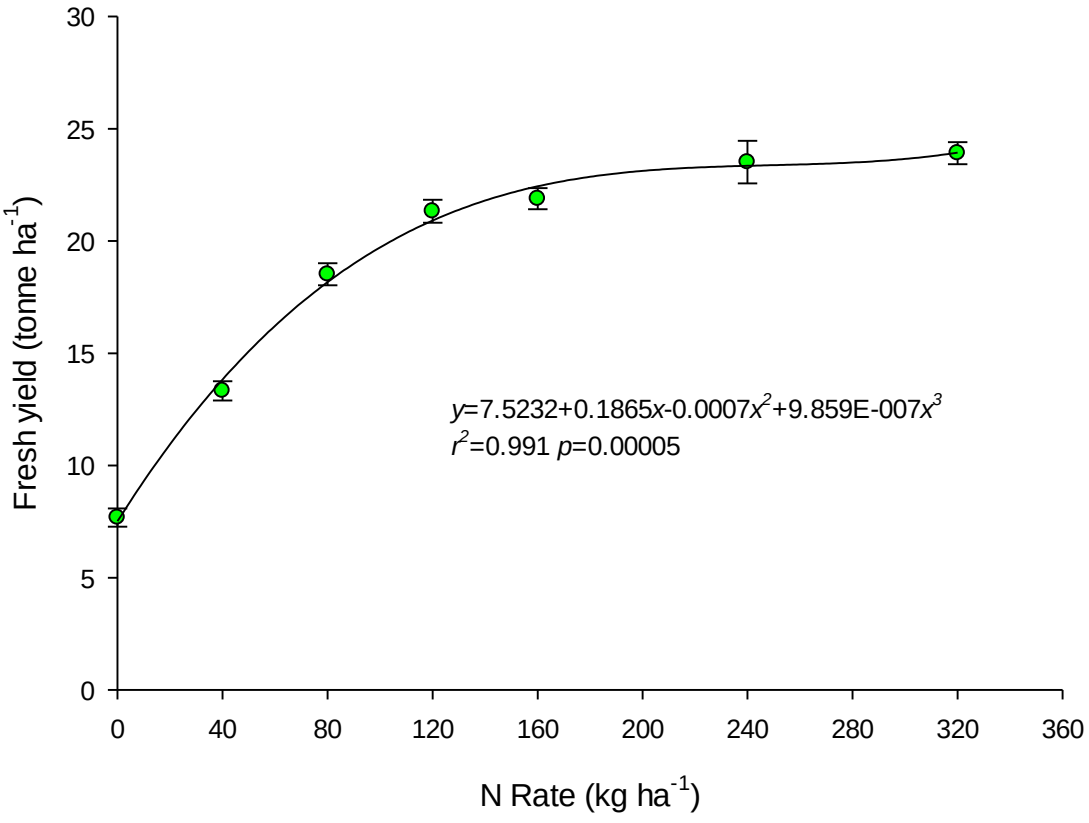


Figure 7.13. Relationship between rate of nitrogen application and shallot bulb yield t ha^{-1} in a trial evaluating effects of nitrogen rate on shallot growth conducted at Cirebon, Java, Indonesia in 2015.

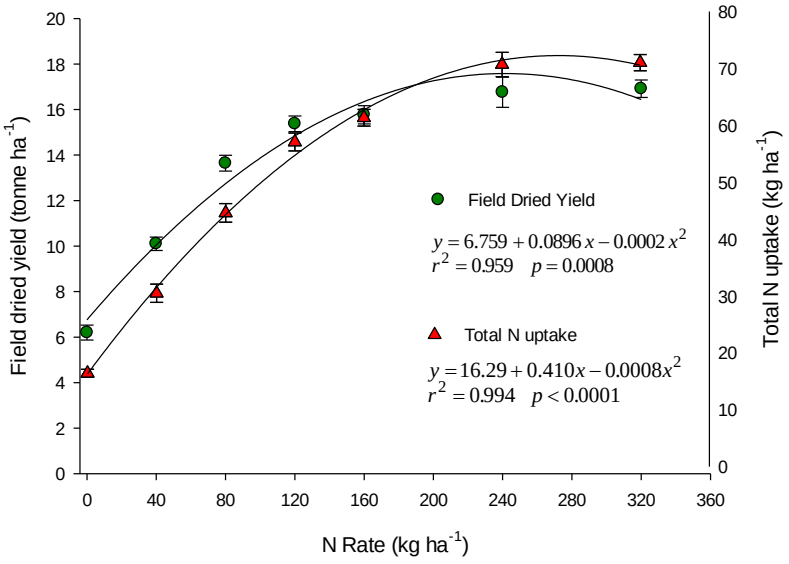


Figure 7.14. Relationship between rate of nitrogen application and shallot bulb yield t ha^{-1} and N application rate and total crop N uptake in a trial evaluating effects of nitrogen rate on shallot growth conducted at Cirebon, Java, Indonesia in 2015.

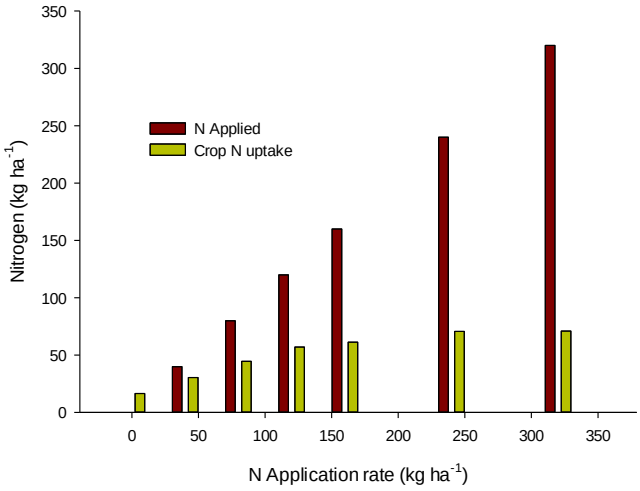


Figure 7.15. Comparison of N units applied as fertilizer (kg ha^{-1}) and total crop N uptake (kg ha^{-1}) in a trial evaluating effects of nitrogen rate on shallot growth conducted at Cirebon, Java, Indonesia in 2015.

Figure 7.16. Shallot growth responses in field (a) and plants (b) in a nitrogen rate response experiment (0, 40, 80, 120, 160, 240 and 320 kg N ha^{-1}) conducted in Java 2015.

7.3.2 Garlic productivity improvements in Australia

Garlic nitrogen trials Australia

A nutrient budgeting exercise was conducted in Queensland to identify the potential mineral nutrient demand in garlic. To achieve this the largest bulbs were selected in 2012 from a commercial growers field known for growing quality garlic. The largest bulbs selected were of an average size of about 80g and considerably larger than other commercial garlic. The samples of garlic were dried and a full nutrient analysis conducted. Nutrient removal was calculated on the basis of 200,000 plants ha^{-1} and a target of an 80g bulb size (16 t ha^{-1}) and a 100g bulb size (20 t ha^{-1}). The data from the nutrient budget are included in Table 7.28 and this identified a target N application rate of 200-250 kg N ha^{-1}.

Table 7.28 Target yields and predicted nutrient removal for garlic grown in Queensland Australia.

Target yield (t ha^{-1})	Nitrogen kg/ha	Phosphorus kg/ha	Potassium kg/ha	Sulfur kg/ha	Calcium kg/ha	Magnesium kg/ha
16	206	22	46	38	17	4
20	257	27	58	48	22	5
	Manganese g/ha	Iron g/ha	Copper g/ha	Zinc g/ha	Molybdenum g/ha	Boron g/ha
16	493	4618	175	1747	9	624
20	616	5772	218	2184	12	780

An experiment based on these rates was conducted in 2014 but did not show a significant bulb yield or bulb-size response to N application rates of 0-200 kg N ha^{-1}; yields ranged from 6.1-6.7 t ha^{-1}. However, in other agronomic trials for that year and conducted in the same experimental block using the same planting material the yield of garlic across the other trials was substantially greater (8.7 t ha^{-1} at 130 kg applied N ha^{-1}) than the highest yield in the N response trial (6.7 t ha^{-1} at 120 kg N ha^{-1}) (Figure 7.17) and the other agronomic trials visually looked healthier. The only management difference between the agronomic trials and the N response experiment was that the agronomic trials had N supplied as a balance of nitrate and ammonium forms while urea was used as the sole source of N in the N response experiment. This suggested that the application of N as urea alone did not provide a growth response and appeared to suppress yield.

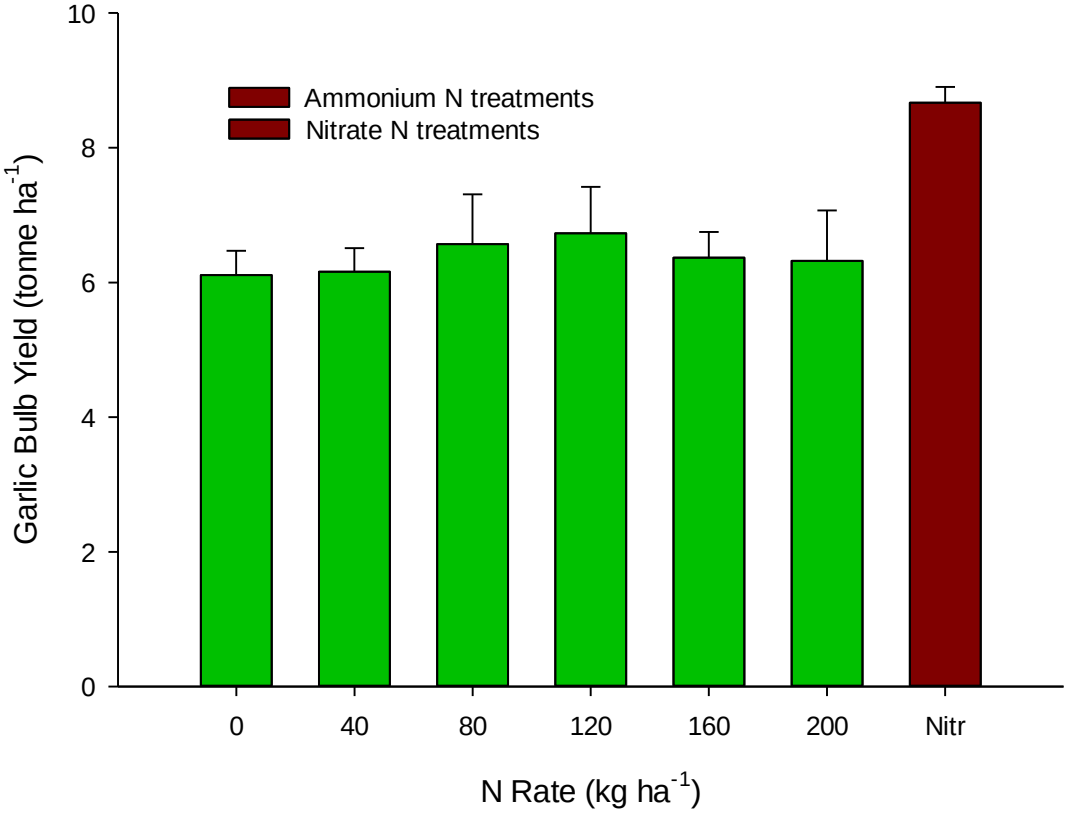


Figure 7.17. Relationship between rate of nitrogen application (0-200 kg N ha^{-1}) and garlic cv. Glenlarge bulb yield t ha^{-1} compared with the mean yield of other experiments conducted at the same time and (▮) at Queensland Government DAF Gatton Research Facility Gatton, Queensland Australia in 2014.

The experiment was repeated in 2015 using nitrate N forms with N rates from 0 to 240 kg ha^{-1}. Increasing N rate gave higher bulb fresh and dry matter yield, foliage dry matter yield and total dry matter yield (Table 7.29). The maximum yield was 12.1 t ha^{-1} at 240 kg N ha^{-1}. In support of this finding Fernandes *et al.* (2010) found the maximum yield of virus free garlic cv. Hunter (8.9 t ha^{-1}) was recorded at 240 kg ha^{-1}. Trani *et al.* (2008), in two seasons found that maximum garlic yields of 3.9 and 8.7 t ha^{-1} were obtained at 74 and 107 kg N ha^{-1} respectively. Increasing N application to 240 kg ha^{-1} also gave the greatest mean bulb weight (62.5 g). However, in contrast to the yield data the highest DM% was recorded in the 0 kg N ha^{-1} treatment (45.2%) and decreased with increasing N application to a minimum of 42.5% at 240 kg N ha^{-1}.

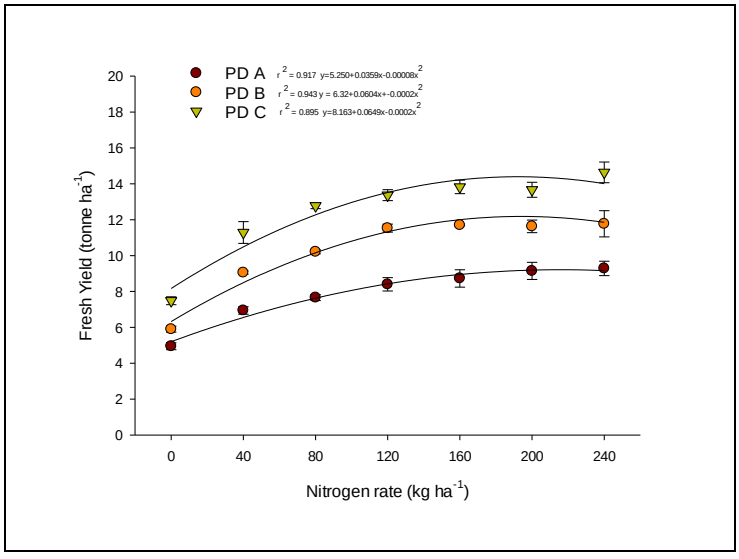
The experiment also evaluated the interaction between plant density and N rate. Averaged across N rates, all of the yield components (Bulb fresh yield, bulb dry matter yield, Foliage dry matter yield and total crop dry matter yield) increased with increasing plant density as a function of a higher plant number per ha (Table 7.30). However, the mean bulb weight declined substantially with an increasing plant density. At a plant density of 266,000 plants ha^{-1} the bulb weight was only 46.7 g and below an optimal marketable size.

Table 7.29. Effect of N application (kg ha^{-1}) on garlic (cv. Glenlarge) bulb fresh (Bulb Fr Yld) and dry matter (Bulb DMYld) yield (t ha^{-1}), foliage dry matter yield (Fol DMYld), total dry matter yield (Total DMYld), bulb percentage dry matter concentration (Bulb DM%), and mean bulb weight (Mean Bulb Wt) in an experiment conducted at Gatton Qld Australia in 2015.

N Rate (kg ha^{-1})	Bulb Fr Yield	Bulb DMYld	Fol DMYld	Total DMYld	Bulb DM%	Mean Bulb Wt (g)
0	6.1 e	2.76 e	0.82 c	3.58 e	45.2 a	31.5 e
40	9.1 d	3.89 d	1.07 b	4.96 d	43.1 bc	46.5 d
80	10.2 c	4.50 c	1.21 a	5.71 c	44.0 ab	52.1 c
120	11.1 b	4.86 b	1.26 a	6.12 b	43.8 b	56.9 b
160	11.4 b	4.85 b	1.24 a	6.08 b	42.4 c	58.6 b
200	11.3 b	4.78 b	1.21 a	5.99 bc	42.5 c	57.6 b
240	12.1 a	5.17 a	1.30 a	6.47 a	42.5 c	62.5 a
F Prob	<.001	<.001	<.001	<.001	<.001	<.001
LSD =	0.6	0.24	0.09	0.30	1.3	3.3

The interaction between N application rate and plant density was significant for bulb fresh yield, bulb dry matter yield and total dry matter yield (Table 7.30) and a fitted quadratic function showed a high correlation between yield components (Fig. 7.18). The interaction between N application rate and plant density on mean bulb weight was not significant. However, an N rate of 240 kg ha^{-1} combined with a plant density of 200,000 plants ha^{-1} appeared to give the best yield by bulb size combination since the mean bulb size in this treatment (62.4 g) was similar to that recorded for the 120-200 kg N ha^{-1} treatments at 133,300 plants ha^{-1}.

In the 0 N treatment the total crop uptake (above ground biomass) was about 40 kg N ha^{-1} (Table 7.31). On the basis that this amount was supplied at each N treatment the efficiency of N use was below 100% in all treatments where N was applied. At the highest N treatment of 240 kg N ha^{-1} the total plant uptake for foliage and bulb was about 114 kg ha^{-1} indicating a substantial shortfall in N uptake compared with application; notwithstanding further N supply to the crop from the soil labile N pool. Nitrogen uptake and nitrogen fertiliser use efficiency was greater at increasing plant density but across all plant densities nitrogen use efficiency nonetheless decreased with increasing N application rate (Fig. 7.19).



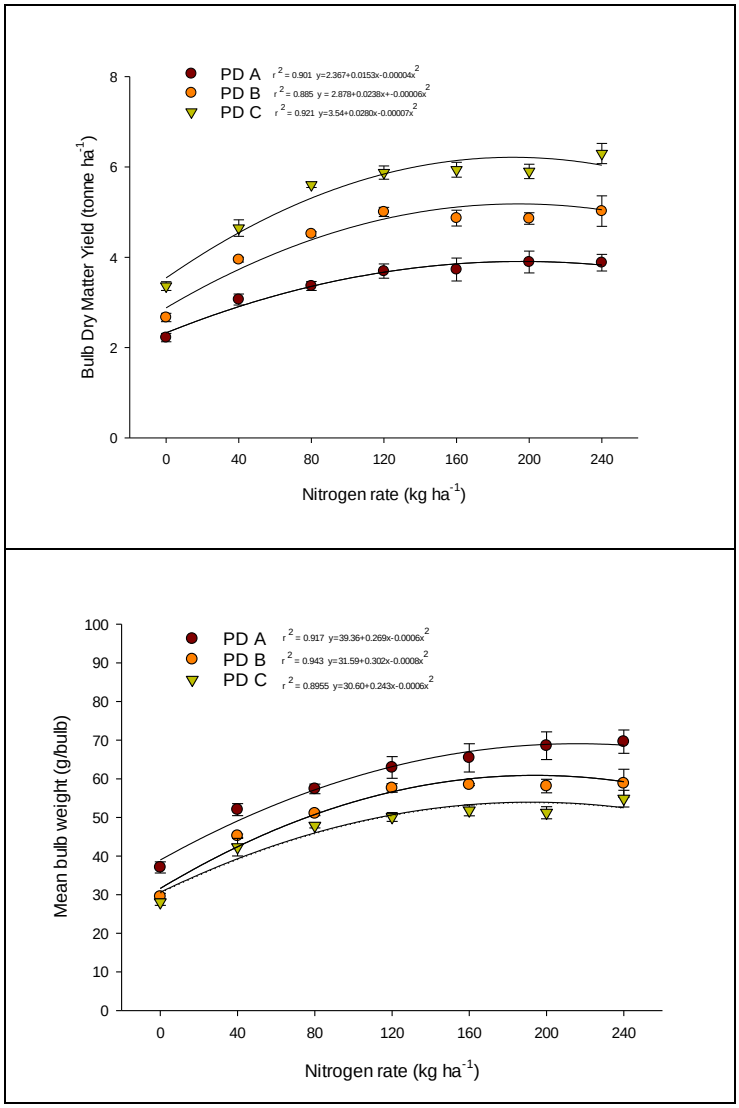


Figure 7.18. Relationship between rate of nitrogen application and garlic (cv. Glenlarge) bulb yield components (Fresh Yield, dry matter yield and mean bulb weight) at plant densities of 133 300 (PD A), 200 000 (PD B) and 266 600 (PD C) plants ha^{-1} in a trial evaluating effects of nitrogen rate on garlic growth conducted at the Queensland Government DAF Gatton Research Facility in 2015.

Table 7.30. Effect of plant density ('000 plants ha^{-1}) on garlic (cv. Glenlarge) bulb fresh (Bulb Fr Yld) and dry matter (Bulb DMYld) yield (t ha^{-1}), foliage dry matter yield (Fol DMYld), total dry matter yield (Total DMYld), bulb percentage dry matter concentration (Bulb DM%), and mean bulb weight (Mean Bulb Wt) in an experiment conducted at Gatton Qld Australia in 2015.

Plant Population ('000 plants ha^{-1})	Bulb FrYld	Bulb DMYld	Fol DMYld	Total DMYld	Bulb DM%	Mean Bulb Wt (g)
133	7.8 c	3.37 c	0.90 c	4.27 c	43.5	58.3 a
200	10.4 b	4.46 b	1.19 b	5.65 b	43.2	51.8 b
266	12.4 a	5.38 a	1.38 a	6.76 a	43.3	46.7 c
F Prob	<.001	<.001	<.001	<.001	0.791	<.001
LSD =	0.4	0.16	0.06	0.20	NS	2.2

Table 7.31. Effect of N application (kg ha^{-1}) on garlic (cv Glenlarge) nitrogen uptake and partitioning (kg ha^{-1}) between foliage and bulbs and difference between applied fertilizer and N uptake (NAppl-NUptk) (kg ha^{-1}) in an experiment conducted at the Queensland Government DAF Gatton Research Facility in 2015.

N rate (kg ha^{-1})	Foliage (kg N ha^{-1})		Bulb N (kg N ha^{-1})		Total N (kg N ha^{-1})		NAppl-NUptk
0	7.0	d	31.5	f	38.4	f	-38.4
40	9.7	c	53.4	e	63.1	e	-23.1
80	12.3	b	66.4	d	78.7	d	1.3
120	12.5	b	79.5	c	92.0	c	28.0
160	11.5	b	89.0	b	100.6	bc	59.4
200	12.4	b	96.2	b	108.9	b	91.1
240	15.2	a	111.2	a	126.4	a	113.6
F test Prob.	<.001		<.001		<.001		
LSD (5%) =	1.71		8.559		8.947		

The highest yield was recorded at an N application rate of 240 kg ha^{-1} and though the increase in yield was more marginal at rates above 120 kg N ha^{-1} and a greater proportion of the applied N was not used by the crop, the increase in yield of about 1 t ha^{-1} (approximately AUD20,000 per ha at a unit price of $20 per kg) provides a return far greater than the cost of the extra N input (approximately AUD150 per ha).

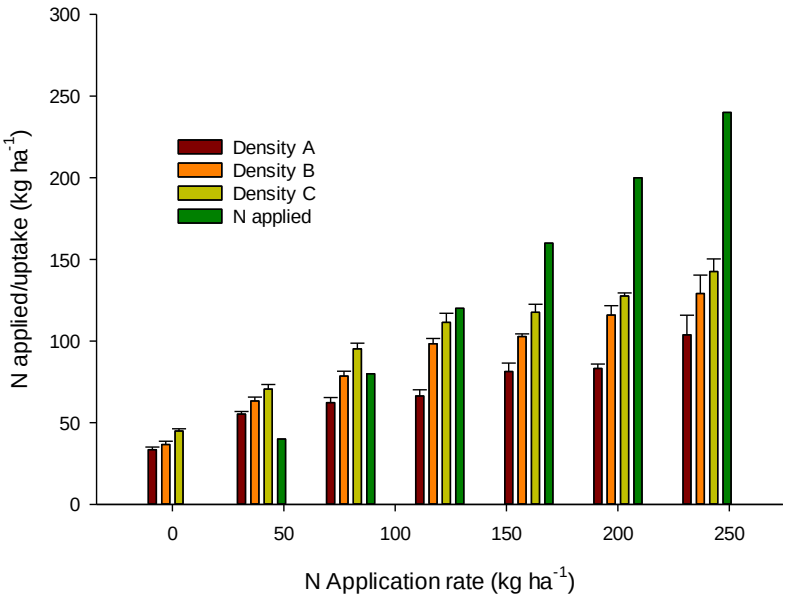


Figure 7.19. Comparison of N units applied as fertilizer (N applied) (kg ha^{-1}) and total crop N uptake (kg ha^{-1}) at 3 plant densities (A 133,300 plants ha^{-1}, B 200,000 plants ha^{-1} and C 266,600

plants ha^{-1}) in a trial evaluating effects of nitrogen rate on garlic (cv. Glenlarge) growth conducted in 2015.

The total N concentration and nitrate concentration in the reference leaf, bulb and foliage at harvest all increased with increasing N application rate (Table 7.32, Fig. 7.20). Both the N and nitrate concentrations in the reference leaf were good indicators of garlic N status. The nitrate and total N values increased substantially from 0-160 kg ha^{-1} then plateaued to give only marginal increases from 160-240 kg ha^{-1}. These coincided with the yield response profile that increased substantially up to 160 kg N ha^{-1} but above which a plateau was observed with reduced yield response up to 240 kg ha^{-1}. In maximizing garlic yield a critical leaf N concentration of about 4.2% or a leaf nitrate content of about 50 mg kg^{-1} is required.

Table 7.32. Nitrate and total N concentrations in a Garlic (cv Glenlarge) reference leaf sample (youngest fully expanded leaf), foliage at harvest and garlic bulbs collected from plants grown under a series of N treatments (kg ha^{-1}) in an experiment conducted in 2015.

N Rate (kg ha^{-1})	Nitrate content (mg kg^{-1})			N content (%)		
	Reference Leaf	Foliage (harvest)	Bulb	Reference Leaf	Foliage (harvest)	Bulb
0	30 e	28 c	10 b	2.70 e	0.86 c	1.15 e
40	34 de	29 c	10 b	3.16 d	0.91 bc	1.40 d
80	41 cd	32 c	10 b	3.57 c	1.01 b	1.49 cd
120	45 bc	33 c	7 c	3.98 b	1.00 b	1.63 c
160	50 ab	54 b	7 c	4.21 a	0.94 bc	1.85 b
200	53 a	54 b	10 b	4.22 a	1.02 b	1.97 b
240	56 a	74 a	12 a	4.31 a	1.15 a	2.22 a
F test Prob.	<.001	<.001	<.001	<.001	<.001	<.001
LSD	7.2	8.3	1.9	0.191	0.11	0.139

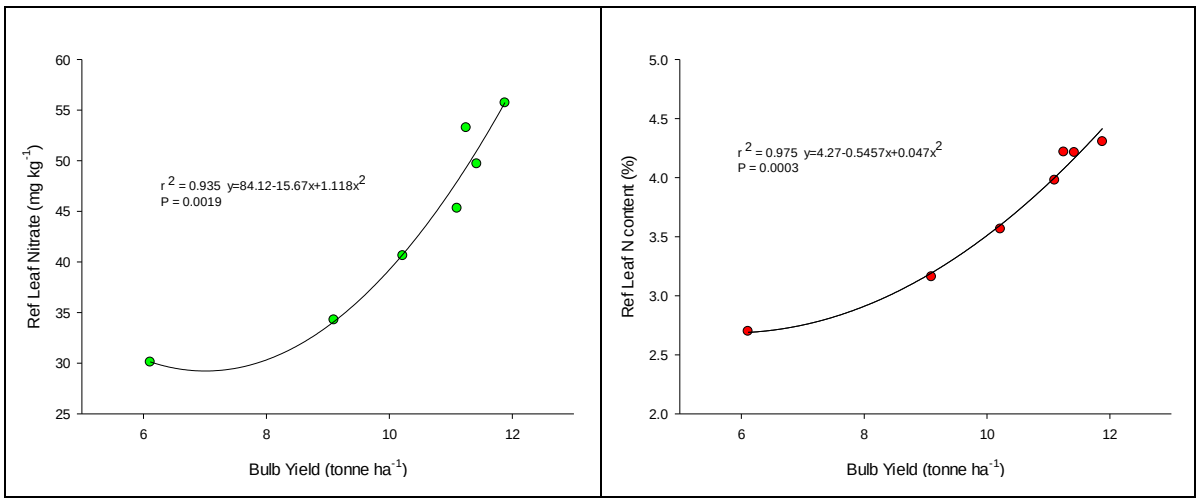


Figure 7.20 Relationship between leaf nitrate concentration (left) and dry tissue total N concentration in garlic (cv. Glenlarge) in a trial evaluating effects of nitrogen rate on growth conducted in 2015.

The effect of seed crop nutrition was evaluated by replanting the bulbs retained from each treatment in the 2015 N response experiment and planted these in 2016 at a uniform rate of N of 180 kg N ha^{-1}. The data showed three levels of response that highlighted the lowest yield at the 0N kg ha^{-1} seed, a second tier response at 40 to 120 kg N ha^{-1} and the highest yield was recorded in the seed from the 160-240 kg N ha^{-1} treatment (Fig 7.21). This data highlighted that, in garlic, where saved vegetative seed is planted the following season, agronomy of the previous season's crop impacted on the growth of the subsequent crop.

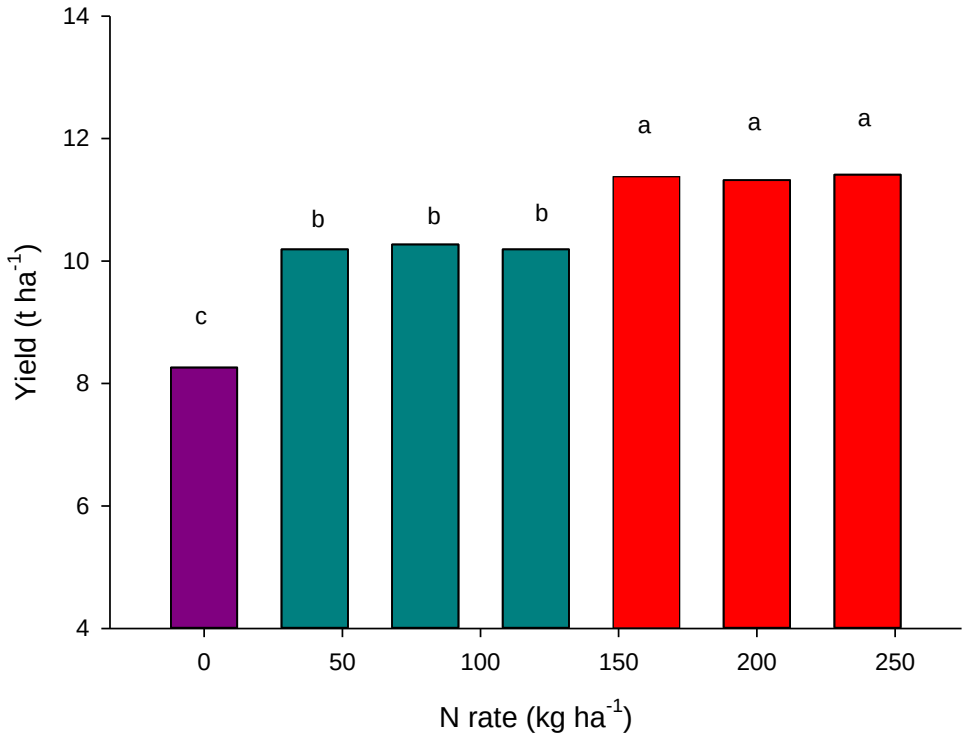


Figure 7.21. Effect of seed crop N status (0, 40, 80, 120, 160, 200 and 240 kg N ha^{-1}) on subsequent garlic crop growth grown with 180 kg N ha^{-1}.

The results of the experimentation on the effects of N on garlic yield highlight that N requirements for maximum yield are in the order of about 200 kg ha^{-1} and are higher than that which the Queensland garlic industry would typically apply (eg. about 100-120 kg N ha^{-1}). A combination of high N application with a planting density of 200,000 plants ha^{-1} gave the highest marketable yield (12.5 t ha^{-1}) and quality. At a lower plant density of 133,300 plants ha^{-1} the yield was nonetheless high at 9.3 t ha^{-1}. The application of N solely as ammonium forms or urea gave lower yields than did nitrate forms of fertilizer but the underlying mechanism behind this requires investigation. The critical N value (%) to achieve maximum yield, determined in the youngest fully expanded leaf at 118 DAP, was about 4.2%. Nitrate concentration expressed on a dry tissue basis (50 mg kg^{-1}) was also a reliable indicator of crop N status.

Effect of N form on garlic growth

Based on the lack of an N response in 2014, an experiment was conducted to evaluate the effect of forms of N fertilizer on garlic growth. The results showed that yield was significantly reduced with application of ammonium fertilizer forms (including urea) or chicken manure (Table 7.33). Application of N in nitrate form increased garlic yield by about 11% in this experiment compared with the other treatments. This data indicates that garlic appears to be sensitive to ammonium forms of N and that the lack of response in the 2014 N rate experiment was likely to be related to the imposition of the N rates in the form of urea. The issue of garlic

sensitivity to ammonium requires further research to understand the underlying mechanisms behind this. However, this is consistent with the results of other research (Gamiely *et al.*, 1991, Woldetsadik *et al.*, 2002) where application of ammonium N alone gave lower yields than with a nitrate form of fertilizer. The study of Woldetsadik *et al.* (2002) showed ammonium alone reduced shallot bulb fresh weight by about 40-50% compared with nitrate forms.

Table 7.33. Effect of different forms (nitrate ammonium and chicken manure) on Bulb fresh yield (t ha^{-1}) and mean bulb weight (g) of garlic (cv. Glenlarge) in an experiment conducted at Gatton, Qld Australia in 2015.

Fertiliser treatment	Bulb fresh Yield (t ha^{-1})	Bulb fresh weight (g)
Nitrate	8.89 a	67.34 a
Chicken manure	8.05 b	60.35 b
Ammonium	8.00 b	59.98 b
Ammonium and nitrate	7.96 b	59.67 b
P =	0.037	0.037
LSD =	0.749	5.619

Agronomic improvements in garlic productivity

Seed selection

A series of experiments evaluated optimized practices for improving garlic productivity. This included processes for reselecting healthier garlic planting seed, herbicide effects and seed planting orientation. The reselection of garlic has been a key component of improving garlic yield. Early in the project, during the 2012 season, healthy vigorous plants were identified from a commercial grower's field, tagged at the active vegetative stage and at maturity harvested and held for planting in 2013. This selection gave a large category of seed (≈80g bulb size) and a further selection of a small category (≈40g bulb size) and a medium category (≈60g bulb size) was also taken. The seed was planted in the following season to compare effects of bulb size on garlic yield. The experiment gave clear visual and yield treatment effects (Fig 7.22). The yield in the plants grown from the large healthier seed was greater than that of either the medium or small seed ($p<0.05$). The healthy seed yield (6.9 t ha^{-1}) was three times the yield of the small seed. Interestingly, in the poor seed, random healthy plants appeared at low percentages (<5%).

The reselection process for seed quality was applied on an annual basis which, along with the improvements in agronomy, have delivered progressive annual improvements in garlic yield. The reselection process has been based on the colour of foliage (a uniform deep blue–green colour), thickness of stem and size of bulbs. Having developed a substantially improved seed line, a further phenotype for selection against virus infection is the presence of white chlorotic lesions on the stem (pseudostem) as plants approach maturity. Figure 7.23 shows a reselected line without lesions (left) and a line with stem lesions plus an inset highlighting the lesions (right). The presence of the lesions appears associated with high virus titre.

Figure 7.22. Effect of seed, selected on a size basis (large, medium and small), on garlic yield.

Figure 7.23. Absence of pseudo-stem lesion in larger garlic cv. Kenlarge (Left) and pseudo-stem lesion development in plants affected by virus (right).

Seed selection based on bulb size was well correlated with crop yield and the regression analysis accounted for about 19% of the variance (Fig 7.24). Recent research on the relationship between individual seed-clove size and bulb yield showed a better correlation (38%) than bulb size alone as a selection criteria. A combination of bulb and clove size may be the best process for seed selection and requires further evaluation.

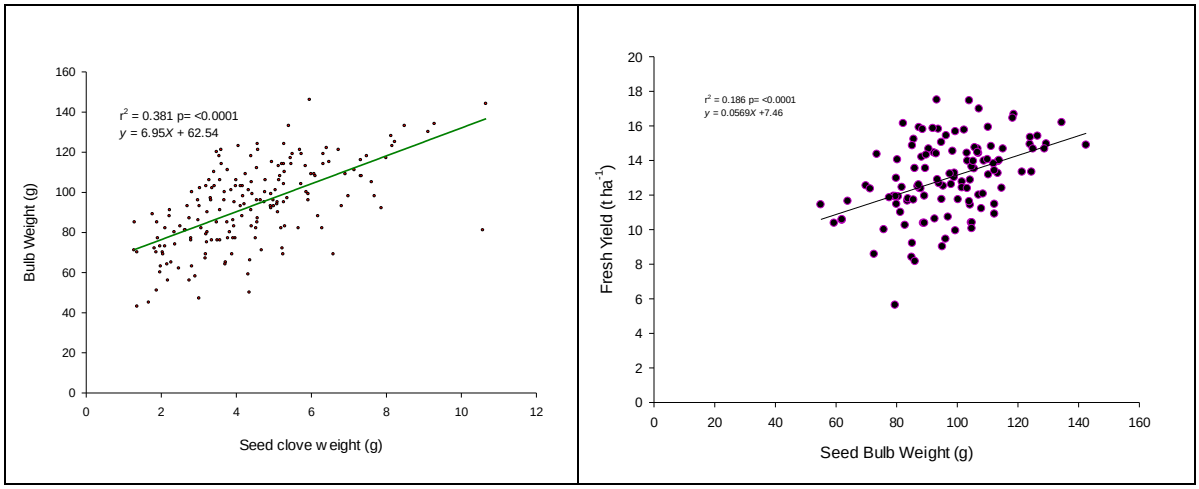


Figure 7.24. Correlation between seed-bulb weight and yield (left) and seed clove weight and yield (right) in garlic cv. Glenlarge.

Table 7.34. Virus status of garlic lines reselected at DAF Gatton Research Facility.

Cultivar	Carlavirus		Potyvirus		Allexivirus					
	GCLV	SLV	OYDV	LYSV	GVA	GVB	GVC	GVD	GVE	GVX
Kenlarge-16-1	-	+	+	+	-	+	+	+	-	+
Glenlarge Standard	+	+	+	+	+	+	+	+	+	+
Glenlarge small	+	+	+	+	+	+	+	+	+	+
Southern Glen	+	+	+	+	-	+	+	+	-	-

The reselection process and the development of improved seed material is related to reductions in virus titre and elimination of viruses. Evaluations of presence or absence of the known viruses highlights that the reselected line of Kenlarge (selected from cv. Glenlarge) has omitted GCLV, GVA and GVE (Table 7.34). The diagnostic test is not quantitative and hence the other viruses may be present at low titer but nonetheless test positive.

Over the course of the project considerable gains in garlic productivity have been demonstrated. The average annual yield improvement in garlic has been about 24% over a 5 year period going from 6.3 t ha^{-1} in 2013 to 17.6 t ha^{-1} in 2018 (Fig 7.25).

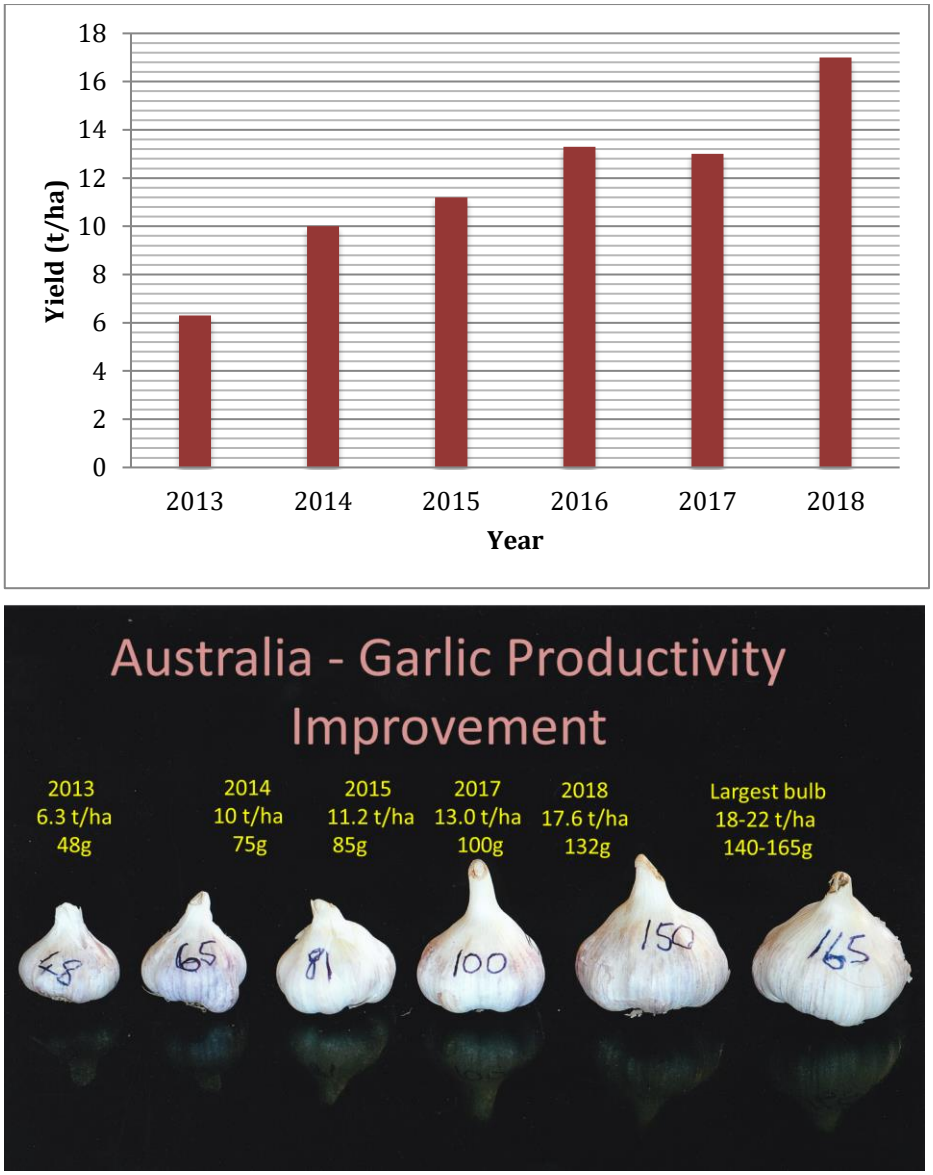


Figure 7.25. Productivity gains in Queensland agronomy trials conducted from 2013 to 2018.

The relative impact of the contribution of fertilizer management and seed selection in improving garlic yields was evaluated. A sequential experiment was conducted over two seasons (2017 and 2018) and compared the effects of seed selection (large seed and small seed) and fertilizer treatments. The seed planted in 2018 was retained from the plots used in the 2017 phase and resown as the same treatments. The fertilizer treatments consisted of a standard garlic grower N rate of 110 kg N ha^{-1} and an improved best management option of 180 kg N ha^{-1} with and without micronutrient application. Seed selection had a substantial effect on garlic growth (Table 7.35). In 2017, the yield from large seed was about 40% greater than for small seed and in 2018 large seed was about 75% greater than for small seed. The relative change in yield between treatments from 2017 to 2018 showed a different response for small and large seed. For large seed there was a relative yield improvement of about 18% from 2017 to 2018 but for the small seed over the same comparison, yield decreased by 5% with the high N BMO and by 15% in the low N grower practice. In both years, where good seed was used, the high N treatment gave about a 1 t ha^{-1} yield improvement over the standard practice (Table 7.35) which was worth about AUD$20,000.

Table 7.35. Effect of seed selection (large and small seed) and fertiliser practice (Best management option – BMO, BMO plus trace element (TE) and standard farmer practice rate) on garlic yield (tonne ha^{-1}) in garlic trials conducted at DAF Gatton Research Facility in 2017 and 2018.

Fertiliser practice	Seed Quality	2017		2018		Relative yield change (%)
BMO	Large	14.95	a	17.4	a	116.4
BMO+TE	Large	14.79	a	17.57	a	118.8
FarmPrac	Large	13.76	b	16.4	b	119.2
BMO	Small	11.81	c	9.98	c	84.5
FarmPrac	Small	10.83	d	9.99	c	92.2
BMO+TE	Small	10.18	d	9.87	c	97.0

Garlic mechanization potential

Planting and weed management are the two key factors that influence the capacity of garlic growers to produce large volumes of garlic. Australian garlic growers use various planting equipment, all of which do not carefully regulate seed orientation. An experiment compared the effect of clove orientation at planting on yield. Cloves were planted either upside down, upright or on their side. The data confirmed that upright planting of the clove gives substantially greater yield and particularly in the large market categories (Table 7.36). The loss in yield of more than 3 t ha^{-1} with poor seed orientation (upside down or sideways) is a major impediment to producing quality garlic with machine planting. A further assessment of herbicide impacts on garlic growth showed a 10% yield reduction with herbicide application (700 g ha^{-1} Methabenzthiazuron plus 700 mL ha^{-1} Ioxynil) (Figure 7.26). Spectral imaging of garlic crops confirmed a high level of crop stress after spraying with herbicide (Fig 7.26).

Table 7.36. Effect of garlic seed bulb orientation at planting (upright, sideways and upside-down on garlic yield (tonne ha^{-1}) in a trial conducted at DAF Gatton Research Facility in 2018.

Seed Orientation	Yield (t ha^{-1})				
	Small (<45mm)	Medium (45-55mm)	Large ^{NS} (55-70mm)	Extra large (>70mm)	Total Yield
Down	0.6 b	2.3 b	6.8	0.2 a	10.0 a
Sideways	0.1 ab	1.4 ab	8.2	3.8 b	13.5 b
Upright	0.0 a	0.5 a	8.8	7.2 c	16.5 c
F test Prob.	0.05	0.018	0.332	0.006	<0.001

^{NS} denotes treatment differences are not significant at 5%

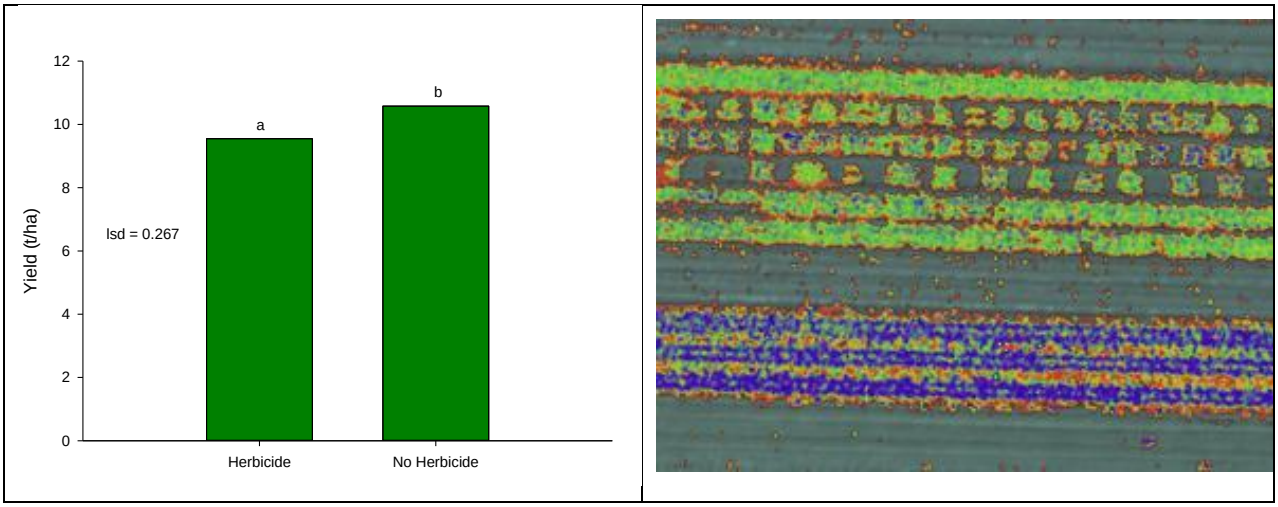


Figure 7.26. Effect of herbicide application on a) yield of garlic (left) and b) spectral reflectance. The lower half of the right image showing a negative effect (blue colour reflectance) compared with the healthy green above.

7.3.3 True Shallot Seed (TSS) - production schemes and improved sowing techniques.

Production of TSS

In Yogyakarta, 3 native shallot cultivars (Tiron, Biru and Crok kuning) and one commercial cultivar (Tuk-tuk) were evaluated for their ability to produce TSS in lowland areas. The four cultivars had 16 chromosomes and karyotyping four 4 chromosome pairs of metasentris, 3 pairs of submetasentris and 1 pair of subtelosentris, so that the formula chromosomes are 3SM + 4M + 1ST. These observations confirm that the tested material tested was of the *Allium cepa* L. Aggregatum group. The three native shallot cultivars had high pollen fertility, but low ovule fertility. Pollen fertility in Crok kuning, Biru and Tiron were 81%, 89% and 95%, respectively. Ovule fertility of Crok kuning and Biru were 46% and 42%, respectively. The seed viability of the three shallot cultivars was low (less than 25.3%).

Rosliani (2013) reported that 2 shallot cultivars (Bima-Brebes and Trisula) when planted in Lembang (a highland area) gave the highest TSS production. TSS of Bima Brebes was 1–1.5 g per planted bulb about 150–225 kg ha^{-1} and Trisula produced 135 kg ha^{-1} of TSS. In 2014, the TSS varieties Bima-Brebes, Trisula and Tuk-tuk were planted in BPTP of Central Java (medium land) to develop mini seed-bulbs. Prayudi et al (2014) reported that the direct seedling of 5 kg TSS Trisula generated 7.5 t ha^{-1} mini bulbs (G0 bulbs). The result was less than that reported for Tuk-tuk and Sanren which at 100 seedling m^{-2} produced 30.9 and 39.7 t ha^{-1} respectively (Brink and Basuki, 2012).

TSS production in the lowland area in Bantul was conducted during the second planting season (May/June-July/August 2018) when lower temperatures are recorded and resulted in limited flower formation and produced some seeds. However, the seeds had very low viability (<20%). In the upland areas where farmers grow shallot during the rainy season floral initiation was good but the prevailing wet conditions did not support pollination and subsequent seed production. TSS production in closed plastic houses with insect pollinators appears to be the best option but nonetheless results in severe infection by Stemphillium sp. and potential spore contamination in the seed.

Improved sowing techniques and transplanting in TSS cultivation

This study aimed to identify which TSS sowing method is most technically and economically feasible. A factorial field trial was conducted in Brebes District, Central Java Province with two factors including sowing methods (S1: evenly spread on sowing media, S2: line-spread and S3: soil-media cake) and sowing age (U) (U1: 30 days and U2: 45 days). Results showed that the vegetative growth of seedlings from the soil-media cake sowing method was consistently better than those of evenly-spread and line-spread sowing methods. The soil-media cake is the most technically feasible sowing method. The number of dead plants during transplanting was also considerably lower for seedlings coming from the soil-media cake method compared with evenly-spread and line-spread sowing methods, since root systems are relatively undisturbed at planting. Plants generated from the soil-media cake sowing method had the highest tuber weight per sample. In contrast, plants generated by evenly-spread and line-spread sowing methods had the highest tuber weight per plant since there is less competition. Seedlings generated from the soil-media cake produced the highest tuber fresh and dry weight compared with those generated from evenly-spread and line-spread sowing methods. The fresh yield of shallot sown using the soil-media cake method was 29.3 t ha^{-1} (15.8 t ha^{-1} after a weighted adjustment for 54% land use efficiency) and the dried (cured bulb) dry yield was 17.7 t ha^{-1} (9.6 t ha^{-1} after a weighted adjustment for 54% land use efficiency).

Based on its highest B/C ratio (0.75) and its highest marginal rate of return, it could be confirmed that the soil-media cake technique & 30 days sowing age is the most economically feasible sowing method. The cost per seedling ranges from IDR 37.6 to IDR 42.6. With a 10 x 10 cm planting arrangement and land efficiency of 65%, the number of required seedlings per hectare is 650,000 seedlings. These will cost about IDR 24.4–27.7 million. Seedling cost of this magnitude is equivalent to the cost needed for purchasing 1.2 tonne of shallot seed bulbs at IDR 20,000 per kg in non-TSS shallot cultivation. This implies there is no advantage of using TSS since the seed cost is not cheaper. Further economic analysis suggests that the average cost of TSS production is about IDR 123.1 million per hectare. With the average yield of 6.7 t ha^{-1} (dry yield), the price break-even-point is IDR 18,245 per kg. The price break even point suggests that the farm will experience a loss if the product selling price is below IDR 18,245 per kg. Even though producing a quite high fresh yield (18-29 t ha^{-1}), a relatively low land efficiency of 54% and high weight loss of 56% have substantially reduced the marketable yield. As a consequence, the probability of experiencing a loss in TSS cultivation is still quite high. Further research directed to lowering seedling production costs, increasing TSS productivity (yield), increasing the land efficiency factor, and reducing the weight or storage loss of TSS bulbs is required.

In Australia, productivity of the East West shallot cvs TukTuk, Sanren and Lokanata has been evaluated using TSS and seed bulb. Propagation using TSS results in considerably lower incidence of seed stems (an undesirable market trait) compared with seed-bulbs.

7.3.4 Chili and capsicum root system improvement and nutrient uptake.

During the course of the project a series of experiments was conducted at the DAF Gatton Research Facility to evaluate the effect on productivity of grafting a standard commercial capsicum (bell pepper) cultivar Warlock over rootstocks of wild *C. chinense.* A small selection of data is presented here.

In Experiment 1 the plant fresh yield data for the Warlock (scion) grafted over rootstocks of accessions 40-4, 40-5, 42-2, 42-3, 45-1, 46-1, 50-5, 55-2, 59-2, 59-8, 70-4 and 71-3 was significantly greater than that for W/W (Table 7.37). However, in contrast the foliage DM% (scion cv Warlock) of these accessions, with the exception of 71-3 was considerably lower (a range of 14.0 to 14.8%) than that of W/W (15.7%). The DM% of 71-3 (15.2%) was similar to that of W/W. The combination of a high DM% and fresh yield gave 71-3 the highest plant dry

matter weight (93.3 g plant^{-1}) which along with 42-2 (79.2 g plant^{-1}) and 40-5 (82.8 g plant^{-1}) were greater than that for W/W (67.3 g plant^{-1}). In subsequent experiments (data not presented) the accessions 40-5, 42-3, 71-1, 71-3 and 45-1 consistently exhibited greater foliage and fruit productivity than W/W. In several experiments the lines 71-1, 71-3 and 45-1 had greater fruit set than W/W. Overall the total plant dry matter per plant for 40-4, 40-5, 71-2, 71-1, 71-3 and 45-1 was greater than that for W/W. The data from Experiment 2 is not included in this report.

For the grafted accessions in Experiment 3 harvest 1 the foliage fresh weight of lines 16-5, 30-7, 40-5, 42-3, 45-1 and 71-3-1 and 71-3-3 were greater than that of W/W. The DM% of the foliage varied across accessions with W/W having the highest foliage DM% and significantly greater than that of 42-3, 45-1, 71-3-1 and 71-3-2. The lines 40-5, 42-3 and 45-1 had foliage dry matter yields greater than W/W. The foliage dry weight values for 71-3-1 and 71-3-3 (44.5 and 44.6 g plant^{-1}) tended to be greater than W/W (40.7 g/plant) and though not significant were close to the 5% significance level.

For Experiment 3 harvest 2 (table 7.38) the foliage fresh weights of 40-5, 42-3, 45-1, 50-8, 71-2, 71-3-1 were significantly greater than that of W/W. Of particular interest was that foliage fresh yield of line 40-5 was greater than that of the sister line 40-6 an effect also observed in Experiment 1. Lines with lower fresh yields (64-7 and W/W) also had relatively higher foliage DM% (14.6 and 14.3% respectively) whereas the lines with higher fresh yields (42-3, 45-1 and 71-3-1) had the lowest foliage DM% (13.5, 13.6 and 13.6% respectively). Notwithstanding these contrasting differences in fresh weight and DM% the lines 42-3, 45-1 and 71-3-1 had foliage dry weights greater than W/W. The lines 50-8 and 71-2 also had foliage dry weights greater than W/W.

With the exception of 69-3 all accessions had fruit fresh weights greater than that of W/W (table 7.38). The fruit DM% was not significantly different across accessions and subsequently the fruit dry weight (g plant^{-1}) reflected a similar trend to that of fruit fresh weight. A major factor influencing the fruit fresh weight was the fruit number per plant. The accessions with high fruit fresh weights had much higher fruit numbers. The fruit set for W/W (5.3 fruit per plant) was much lower than all of the accessions and particularly the high yielding accession 42-3 (8.1 fruit per plant).

Table 7.37. Differences in yield components for capsicum cv. Warlock grafted over a selection of rootstocks of *Capsicum chinense*, (Harvest 1) in a field experiment (Experiment 1) conducted at the Queensland DAF Gatton Research Facility in 2011-12.

Accession	Foliage Fresh Weight (g/plant)		Foliage DM%		Foliage Dry Weight (g/plant)		Fruit Fresh Weight (kg/plant)		Fruit DM%		Fruit Dry Weight (g/plant)		Number of fruit per plant	Average fruit weight (g)		Total Plant Fresh weight (kg)		Total Plant Dry Weight (g)	
16-5	432	hijk	15.0	bcde	64.3	fghij	1.82	abc	7.30	a	133	a	10.0	91	defghi	2.25	abcde	198	bcde
25-5	451	fghijk	15.3	ab	68.9	cdefghi	1.67	cde	6.96	bcdef	116	bcde	8.8	96	bcdefg	2.12	cdef	185	cdefg
30-7	482	cdefghi	15.1	bcd	72.4	bcdefgh	1.66	cde	6.87	bcdefg	114	bcde	9.6	87	fghijk	2.15	cdef	187	cdef
21-10	490	cdefgh	15.1	abcd	74.5	bcdefg	1.56	def	7.17	abc	112	cde	9.0	89	efghijk	2.05	defg	186	cdef
34-5	433	ghijk	14.7	cdefg	63.3	ghij	1.70	abcde	6.90	bcdefg	117	bcde	9.2	93	cdefghi	2.14	cdef	180	cdefg
40-4	542	abcd	14.6	cdefg	78.9	bcd	1.51	ef	6.93	bcdefg	104	e	11.7	83	ijk	2.05	defg	183	cdefg
40-5	586	ab	14.1	gh	82.8	ab	1.67	cde	6.90	bcdefg	115	bcde	7.6	93	cdefghi	2.25	abcde	198	bcde
40-6	387	k	14.7	cdefg	56.5	j	1.61	cdef	7.02	abcdef	113	bcde	9.6	85	hijk	2.00	efg	170	fgh
42-2	547	abc	14.5	defgh	79.2	bc	1.91	a	7.04	abcde	135	a	9.7	99	bcde	2.46	ab	214	ab
42-3	524	bcdef	14.0	h	72.9	bcdefgh	1.67	bcde	6.74	efg	113	cde	9.1	92	cdefghi	2.07	cdefg	177	defg
44-1	398	jk	14.8	bcdef	59.0	ij	1.43	f	7.06	abcde	102	e	8.3	86	ghijk	1.83	gh	161	gh
45-1	531	bcde	14.3	fgh	76.0	bcdef	1.74	abcd	6.60	g	115	bcde	8.4	105	ab	2.27	abcde	191	bcdef
46-1	520	bcdef	14.8	bcdef	77.0	bcde	1.80	abc	7.01	abcdef	126	abc	9.6	96	bcdefg	2.32	abcd	203	abc
50-3	461	efghijk	14.6	cdefg	67.2	defghij	1.42	f	7.18	ab	102	e	8.1	89	efghijk	1.88	fgh	169	fgh
50-5	522	bcdef	14.7	cdefg	76.6	bcde	1.61	cdef	7.07	abcde	113	bcde	9.4	86	fghijk	2.13	cdef	190	cdef
50-8	452	fghijk	14.6	cdefg	65.6	efghij	1.54	def	6.69	fg	103	e	9.3	83	ijk	2.00	efg	169	fgh
51-9	398	jk	14.7	cdefg	58.2	ij	1.63	cdef	7.11	abcd	116	bcde	8.6	95	bcdefgh	2.03	efg	174	efg
55-2	535	abcde	14.4	efgh	76.7	bcde	1.80	abc	6.79	defg	122	abcd	8.8	103	abc	2.33	abc	199	abcd
59-2	519	bcdef	14.3	fgh	73.9	bcdefg	1.73	abcd	6.97	abcdef	121	abcd	9.1	97	bcdef	2.25	abcde	195	bcde
59-8	547	abc	14.1	gh	77.2	bcde	1.67	cde	6.86	bcdefg	114	bcde	9.3	91	defghij	2.21	bcde	191	bcdef
64-7	410	ijk	15.0	bcd	61.2	hij	1.63	cdef	6.89	bcdefg	113	bcde	10.5	80	jk	2.04	efg	174	efg
69-3	467	defghij	14.4	efgh	66.5	efghij	1.20	g	6.85	cdefg	82	f	7.8	79	k	1.67	h	149	h
70-4	508	cdefg	14.3	fgh	72.4	bcdefgh	1.55	def	6.69	fg	104	e	8.5	91	defghi	2.06	cdefg	177	defg
71-3	609	a	15.2	abc	93.3	a	1.89	ab	6.83	defg	129	ab	8.6	112	a	2.50	a	222	a
W-W	431	hijk	15.7	a	67.3	defghij	1.58	def	6.85	cdefg	107	de	8.0	101	abcd	2.01	efg	175	efg
F Prob.	P<0.001		P<0.001		P<0.001		P<0.001		P=0.007		P<0.001		P=0.218	P<0.001		P<0.001		P<0.001	
LSD =	75		0.63		11.8		0.22		0.33		16			11.1		0.27		24	

NB: Means with same subscript are not significantly different at the P = 0.050 level

Table 7.38. Differences in yield components for capsicum cv. Warlock grafted over a selection of rootstocks of *Capsicum chinense*, (Harvest 2) in a field experiment (Experiment 3) conducted at the Queensland DAF Gatton Research Facility in 2013-14

Accession	Foliage Fresh Weight (g/plant)		Foliage DM%		Foliage Dry Weight (g/plant)		Fruit Fresh Weight (kg/plant)		Fruit DM%	Fruit Dry Weight (g/plant)		Number of fruit per plant		Total Plant Fresh (kg)		Total Plant Dry Weight (g)	
16-5	390	cdef	14.0	bcdef	54.5	cdef	957	abcd	6.2	120	a	7.6	abc	1.36	abcde	174	ab
30-7	387	cdef	14.1	bcd	54.5	cdef	978	ab	6.0	118	ab	7.7	abc	1.38	abcde	173	ab
40-5	419	bcd	13.8	def	57.5	abcd	955	abcd	6.0	115	ab	7.7	abc	1.40	abcd	173	ab
40-6	373	efg	14.0	bcdef	52.2	def	870	bcdef	6.1	105	abcd	6.5	de	1.26	efg	158	bcd
42-3	468	a	13.5	f	63.1	a	960	abcd	6.2	117	ab	8.1	a	1.45	a	180	a
45-1	460	ab	13.6	def	62.2	ab	961	abc	6.0	115	ab	7.2	abcd	1.44	ab	177	a
46-1	373	efg	14.0	bcdef	52.2	def	923	abcde	6.2	114	ab	7.5	abcd	1.31	bcdef	166	abc
50-8	421	bc	14.1	bcd	59.3	abc	837	ef	6.5	108	abc	7.6	abc	1.27	defg	168	abc
55-2	403	cdef	14.0	bcde	56.3	bcd	1016	a	5.7	115	ab	7.9	ab	1.43	abc	172	ab
64-7	335	g	14.6	a	48.8	f	845	def	6.1	102	bcd	6.7	cde	1.20	fgh	151	cd
69-3	377	cdefg	14.0	bcde	52.7	def	768	fg	6.0	90	de	7.1	abcd	1.16	gh	142	de
71-2	408	cde	13.8	cdef	56.5	bcd	879	bcdef	6.1	106	abcd	7.3	abcd	1.31	cdef	162	abc
71-3-1	412	cde	13.6	ef	55.8	cd	894	bcde	6.0	108	abc	7.0	bcd	1.32	bcdef	164	abc
71-3-2	376	defg	14.0	bcde	52.5	def	912	abcde	6.1	111	abc	7.3	abcd	1.32	abcdef	164	abc
71-3-3	392	cdef	14.1	bcd	55.0	cde	958	abcd	6.1	117	ab	7.4	abcd	1.37	abcde	172	ab
W/W	360	fg	14.3	abc	49.6	ef	708	g	6.0	85	e	5.3	f	1.11	h	132	e
Warlock	401	cdef	14.4	ab	56.9	bcd	853	cdef	5.6	95	cde	5.8	ef	1.27	defg	152	cd
F Prob.	P<0.001		P=0.002		P<0.001		P<0.001		P=0.367	P<0.001		P<0.001		P<0.001		P<0.001	
LSD =	45		0.48		6.1		116			16.5		1.1		0.13		18	

NB: Means with same subscript are not significantly different at the P = 0.050 level

Table 7.39. Differences in yield components for capsicum cv. Warlock grafted over a selection of rootstocks of *Capsicum chinense*, (Harvest 3) in a field experiment (Experiment 3) conducted at the Queensland DAF Gatton Research Facility in 2013-14

Accession	Foliage Fresh Weight (g/plant)		Foliage DM%	Foliage Dry Weight (g/plant)		Fruit Fresh Weight (kg/plant)		Fruit Marketable weight (kg/plant)		Fruit DM%	Fruit Dry Weight (g/plant)		Marketable fruit (#/plant)		Total Plant Fresh (kg)		Total Plant Dry Weight (g)	
16-5	429	efg	15.7	67.2	efg	1.28	bcde	1.23	cdef	7.2	92.1	bcde	8.4	a	1.71	cde	159	bcd
30-7	386	g	16.1	61.7	g	1.27	cde	1.22	def	7.3	92.9	bcd	7.9	abc	1.65	def	155	cd
40-5	484	bcd	15.4	74.8	bcd	1.35	abc	1.32	abcd	7.1	95.7	bc	8.2	ab	1.84	abc	170	bc
40-6	406	fg	15.6	63.3	fg	1.30	bcd	1.23	cdef	7.7	98.9	b	7.8	abc	1.71	cde	162	bcd
42-3	570	a	14.7	83.4	a	1.36	abc	1.32	abcd	6.9	94.0	bc	8.1	abc	1.93	a	177	ab
45-1	507	bc	15.3	77.3	abc	1.08	f	1.04	g	6.8	73.4	de	6.3	def	1.59	def	151	cd
46-1	469	bcde	15.5	72.4	cde	1.41	ab	1.37	abc	6.7	94.0	bc	7.6	abc	1.88	ab	166	bc
50-8	512	b	15.8	80.7	ab	1.19	def	1.12	efg	6.6	78.2	cde	7.4	abcd	1.70	cde	159	bcd
55-2	471	bcde	15.2	71.8	cde	1.38	abc	1.36	abcd	6.7	92.1	bcde	7.8	abc	1.85	abc	164	bcd
64-7	386	g	16.1	62.1	g	1.28	bcde	1.25	bcde	7.0	90.3	bcde	7.7	abc	1.66	def	152	cd
69-3	430	efg	15.5	66.7	efg	1.14	ef	1.09	fg	6.8	77.1	cde	7.2	bcd	1.57	ef	144	d
71-2	448	def	15.7	70.0	def	1.30	bcd	1.28	abcd	6.8	90.3	bcde	7.9	abc	1.75	bcd	160	bcd
71-3-1	489	bcd	15.4	74.5	bcd	1.46	a	1.40	ab	8.0	119.7	a	8.2	ab	1.95	a	194	a
71-3-2	482	bcd	15.3	73.5	cde	1.26	cde	1.23	cdef	6.9	86.0	bcde	7.0	cde	1.74	bcd	159	bcd
71-3-3	428	efg	16.1	68.1	defg	1.42	ab	1.41	a	6.5	93.1	bcd	8.3	a	1.85	abc	161	bcd
W/W	448	def	16.0	71.1	cde	1.05	f	1.02	g	6.9	72.0	e	5.8	f	1.50	f	143	d
Warlock	465	cde	15.4	71.9	cde	1.25	cde	1.24	cdef	6.7	84.0	bcde	6.0	ef	1.72	bcde	156	bcd
F Prob.	P<0.001		P=0.161	P<0.001		P<0.001		P<0.001		P<0.337	P=0.003		P<0.001		P<0.001		P=0.005	
LSD =	44.7			7.1		0.15		0.152			20.3		1.1		0.17		23	

NB: Means with same subscript are not significantly different at the P = 0.050 level

At harvest 3 (table 7.39) the foliage fresh weights of 42-3, 45-1 and 50-8, were significantly greater than that of W/W, a result consistent with harvest 2. However, in contrast to harvest 2, the foliage fresh weight values for 40-5, 71-2, 71-3-1 were not significantly greater than that of W/W; though the values *per se* were higher. As for Harvest 2, the foliage fresh yield of line 40-5 (484 g per plant) was greater than that of the sister line 40-6 (406 g per plant).

In contrast to Harvest 2 results, the differences in grafted accession foliage DM% was not significant, Though consistent with harvest 2, the DM% for W/W foliage (16.0%) tended to be higher than that of the majority of accessions.

The highest fresh foliage yield was recorded in accession 42-3 (570 g per plant). It had the lowest DM% value (14.7%) which was consistent with the observation in harvest 2 that lines with lower fresh yields had relatively higher foliage and *vice versa*. Consistent with the results from harvest 2, with the exception of 69-3, 50-8 and 45-1, all accessions had fruit fresh weights greater than that of W/W and subsequently the fruit dry weight (g/plant) reflected a similar trend. As in harvest 2, the major factor influencing the fruit fresh weight was the fruit number per plant and accessions with high fruit fresh weights had much higher fruit numbers. The fruit set for W/W (5.8 fruit per plant) in harvest 3 was much lower than all of the accessions and particularly the high yielding accessions 42-3, 71-3-1, 71-3-3 and 40-5 (8.1-8.3 fruit per plant). Overall the total dry weights for the elite lines 42-3 and 71-3-1 (177 and 194 g per plant respectively) were considerably greater than that of W/W (143 g per plant) as were the dry weights for 40-5 and 46-1 (170 and 166 g per plant respectively). The fruit DM% was not significantly different across accessions.

The research has identified variability in plant productivity of bell pepper cv. Warlock grafted as a scion over rootstocks of accessions of *C. chinense*. In particular the 40-5, 42-2, 42-3, 45-1 and 71-3 series have consistently given greater plant growth. This trait is important for capsicum where a large plant canopy can protect fruit from sunburn damage a major problem in bell pepper production. Of particular interest was that Warlock grafted over the elite lines exhibited higher numbers of fruit compared with the W/W.

The foliage DM% of the W/W is consistently greater than that of warlock grafted over these elite accessions. This could suggest that the uptake and conductance of water to the foliage could be a limitation in the root system of cv. Warlock. This is loosely supported in data that has compared photosynthesis and physiological parameters of the different grafted lines using a Licor photosynthesis analyser. When grown in soil under glasshouse conditions or in field conditions the W/W exhibits lower stomatal conductance than the elite lines (Fig. 7.27). However, when grown in solution culture it has relatively high stomatal conductance. Further microscopy research is underway to evaluate the size and number of xylem vessels in the warlock and *C chinense* accession root systems. This research has the potential to greatly improve the genetic basis for crop adaptation to field stress. In particular, for Indonesia, the onset of severe drought in late season production is a major chilli crop constraint for which germplasm with improved water use efficiency could deliver considerable productivity improvement.

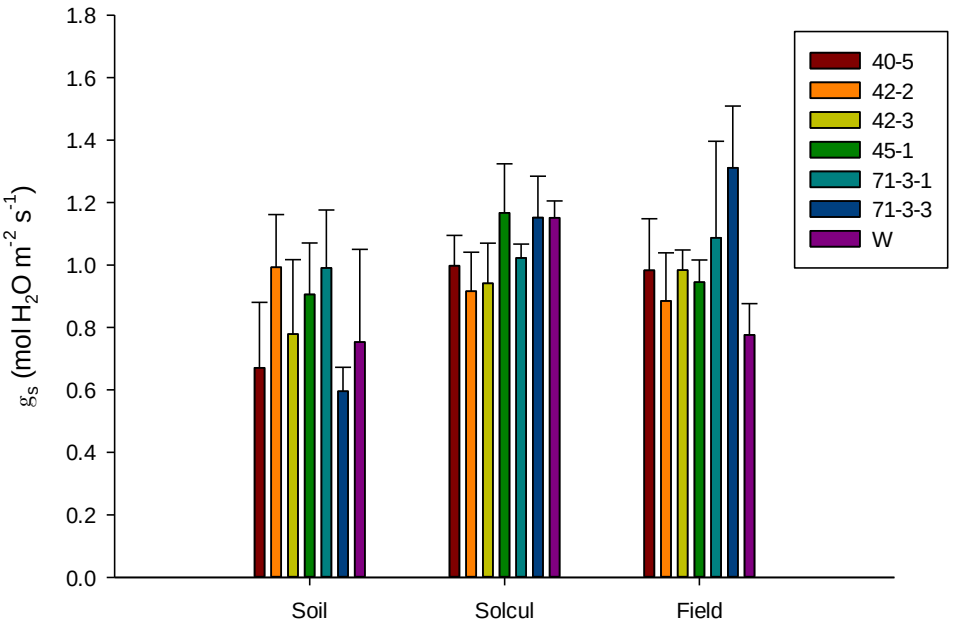


Figure 7.27. Difference in stomatal conductance for grafted capsicum plants (warlock (W) as the scion) and selected *C chinense* accessions and Warlock as the rootstocks grown under greenhouse (soil or solution culture - Solcul) or field conditions.

The line 40-6 was a sister line to 40-4 and 40-5 but had consistently exhibits lower yield values. Similarly, in the 71 series of selections, the 71-2 line tends to have lower productivity that the sister lines 71-3 and 71-1. This suggests that there may be reasonably well heritable traits underlying the identified improvements. These lines are of interest from assessing heritability traits however, considerable research is first required to identify the underlying traits that infer improved productivity.

8 Impacts

8.1 Scientific impacts – now and in 5 years

A major achievement of the project has been the development of molecular probes to differentiate individual virus species of alliums (garlic and shallot). Each of the three main virus genera provides accurate diagnostics to advance the understanding of the spectrum and distribution of allium viruses in Indonesia and Australia. The development of these diagnostics was a substantial component of the project and a major breakthrough in virus identification in alliums. The underlying need for the assessment of viruses in shallot was due to the strong potential for viruses to negatively impact on crop productivity, since shallot is vegetatively propagated. The probes allow the identification of the viruses in the Carlavirus genus (Garlic common latent virus and Garlic latent virus), Allexivirus genus (Garlic virus A, Garlic virus B, Garlic virus C, Garlic virus X and Shallot virus X) and the Potyvirus genus (Onion yellow dwarf virus, Shallot yellow stripe and Leek yellow stripe virus). The application of these diagnostics to field samples of Allium spp. collected in Australia and Indonesia has identified new virus records for both countries, including garlic virus E and a further virus for which the diagnostics are still in progress. These results have greatly enhanced our knowledge of allium viruses and their regional distribution in Australia and Indonesia.

Fusarium disease is a major constraint to shallot production in Indonesia. The UGM research identified that Fusarium (Moler disease) has caused a complex of three different species and includes *F. solani* (the most common species found in all the main shallot production centers), *F. accutatum*, and *F. oxysporum*. This complex of pathogens causes the symptom of wilting often commencing with twisted leaves. Further research has phenotyped the expression of the disease to identify the symptoms associated with single fusarium species infections and as combinations of the Fusarium species. The impact of this is that the research has given a better understanding of the disease complex and an improved screening procedure for identifying resistance that will be adopted by other Indonesian research agencies.

The cause of a prevalent and serious leaf blight disease in onion and bunching shallots in south Queensland has been identified under this project. The research resulted in a world first detection of the bacterial pathogen, *Pseudomonas syringae pv. Porri* infecting onion and bunching onion. Previous detections of the disease has been limited to leeks and this new finding extends the range of susceptible species.

The project partners have evaluated a range of techniques to remove viruses from garlic and shallot germplasm. This research has focussed on tropical shallot and garlic varieties, which builds on existing information in the literature that mostly focuses on temperate germplasm. Workshops at the IHC2014 highlighted that the research on tissue culturing garlic is focussed on long-day temperate varieties so the findings on tissue culturing are novel for the short-day varieties grown in the tropics.

Accessions of *C. chinense* with the ability to deliver superior yield of grafted bell capsicum have been identified, providing the potential to improve the performance of commercial bell pepper cultivars in Australia and to identify drought tolerant lines of chilli for Indonesian production.

8.2 Capacity impacts – now and in 5 years

Training in nutrient budgeting and soil and plant tissue sampling was conducted with the Indonesian teams (UGM and IVEGRI) to develop skills and methodology in evaluation of crop nutrient use efficiency, which can be broadly applied into other crops.

Training was conducted with the project partners from IVEGRI, UGM and IPB in molecular detection and identification of viruses affecting allium crops. This training provided a sound base in molecular biology techniques, which is highly desirable in any institute working in plant pathology research. These skills are not only applicable to the current project but are also transferable to many other crop-virus scenarios. The diagnostics for garlic viruses have been developed and extended to Indonesian scientists and are being applied to shallot and garlic crops.

Since viruses are a critical production issue in garlic and shallot, the development of tissue culture expertise was an essential step in developing clean planting material and increasing crop yields. An important outcome from this project was to instigate research leading to identification of virus free shallot or garlic and developing virus free material using tissue culture. At the initiation of the project there was no active Allium tissue culture in either Australia or Indonesia. Basic skills in virus elimination and tissue culture methodology needed to be developed and implemented. The first step towards building capacity in Allium tissue culture was a very successful first workshop that was held at IVEGRI- Lembang, Indonesia (March 31 to April 1 2014). The intention of the workshop was to formalise the research approaches needed in each organisation so that allium tissue culture systems could be developed and established by all groups. The tissue culture workshop was to consolidate the necessary tissue culture skills and form strong connections between researchers and develop a targeted research approach for the micropropagation and elimination of viruses from selected Allium lines.

This workshop activity included an open sharing of tissue culture and virus experience and research findings to allow the project members to progress work in shallot or garlic tissue culture with the aim of virus elimination. A total of 21 research collaborators from all organisations attended the first workshop. A second tissue culture workshop was held at Bogor Agricultural University, Bogor, Indonesia from 3rd–4th November 2015, Research teams presented their research programs and discussions were held on the success of various practices in removing viruses and producing viable propagules. All collaborators had initiated allium tissue culture research and undertaken virus indexing so significant progress was made over the course of the project. The International Society for Horticultural Science Congress in Brisbane (IHC2014) provided the opportunity for the project team to engage with several international scientists working in garlic and particularly tissue culturing. All research in this discipline has been conducted on European cultivars, adapted to long day lengths and little, if any, work has been done on the day-neutral tropical and subtropical cultivars. There was consensus that only certain cultivars are amenable to tissue culture and others are recalcitrant. In the past year each group has developed some capacity and are advancing their skills in the discipline to the point where culturing is improving.

Advanced training of an Indonesian virologist in molecular diagnostics has improved capacity for the partner country in this area. Furthermore, survey methods have been discussed, developed and implemented with international collaborators to ensure good publishable research outcomes are achieved. The dot blot hybridization method, developed for detection of viruses in shallot and garlic samples, has been successfully adopted by the IPB virology group and provided consistent accurate results. The capacity of the UGM and IVEGRI partners was further developed with IPBs assistance, such that each group now obtains consistency in their diagnostics. This ensures that in the future, valid data comparisons can be made across groups and importantly across regions from where samples are taken.

Under the project, Universitas Gadjah Mada had 43 students complete studies (4 PhD, 26 masters and 13 honours students) linked into the shallot chilli research and experimental programs. These were across the disciplines of socioeconomics, crop nutrition, agronomy and crop pathology. IPB has six students (masters and honours) directly involved in the project and have run experiments as part of their thesis final project. This involvement in the project has given the students opportunities to broaden their knowledge and skill base. Furthermore, collaboration with a broad range of Indonesian and International agencies has been important in developing good networks and providing opportunity to young scientists.

During the course of the project Dr John Thomas, Dr Stephen Harper and Dr Cherie Gambley held focussed discussions with a large number of postgraduate students (≈20 students in total). Students presented their research outline and methodology as a formal presentation in English and specific comments and feedback were provided to identify problems with concept development, methodology and associated issues. The Australian team members are acting in the capacity of secondary advisers for some of these students across different disciplines to improve the rigour of the student's experimentation and they have also sat on review panels examining student's postgraduate programs.

Several Indonesian scientists and post graduate students worked in the field surveying exercise of chilli viruses (Indonesia April 2014) that included the international expertise of AVRDC, DAF and QAAFI staff from which valuable experience and knowledge was gained in field symptom diagnostics, survey techniques and virus epidemiology.

In November 2013 DAF, IVEGRI, IPB and UGM team members visited the Probolinggo region in the far east of Java that is renowned for high quality shallot bulb production. Formal linkages were established between the project staff and the BPTP and University of Surabaya pathologists, which will be further developed in future activities. These relationships will allow better extension of project outcomes to important shallot chilli production areas not covered under the project.

A protocol on fusarium screening has been developed by the UGM team and this has been adopted by the IVEGRI pathology team to screen fusarium resistance in commercially available Indonesian shallot varieties.

Furthermore, through this project Professor Neal Menzies, as Head of The School of Agriculture and Food Science for The University of Queensland, has developed a Memorandum of Understanding for cooperation between UQ and UGM. This linkage is fostering formal student and scientist exchange programs and currently two students from UGM are conducting their postgraduate studies at the University of Queensland.

A training of the Trainer (ToT) program was developed and extended under the project. The aim of the program was to enhance participants understanding of integrated crop management on shallot and chili pepper. It also provided them with current best practice reference information (including project outcomes) on integrated crop management of shallot and chili pepper. The ToT in Sulawesi involved 30 participants (15 extension workers, 10 pest observers and 5 progressive farmers) recommended by the Regional Agricultural Office. Participants came from six sub-districts including Enrekang District, i.e. Baraka, Malua, Anggeraja, Enrekang, Maiwa, and Baroko. A further ToT was held in Probolinggo (March 2019). In coordination with the Probolinggo District Agricultural Office and East Java Assessment Institute of Agricultural Technology, 34 participants attended a training of trainer (ToT) program. The ToT participants included 25 extension workers and 9 staff of the Probolinggo District Agricultural Office. The extension workers were equally selected from 15 sub-districts (Sukapura, Sumber, Kuripan, Bantaran, Leces, Tegalsiwalan, Banyuanyar, Tiris, Krucil, Gading, Pakuniran, Kotaanyar, Paiton, Besuk, and Kraksaan) in which they were assigned.

8.3 Community impacts – now and in 5 years

The project team in Indonesia has engaged and consulted widely with farming communities in the shallot production areas of Cirebon, Brebes, Nganjuk and Bantul to evaluate issues around chemical use, pest management, crop and soil management, seed supply systems, varieties and other production information, as well as supply chains and marketing. This has been conducted as a wide-ranging survey consultation across the farming communities and shallot traders and has provided an opportunity to outline the aims of the project at a farmer and community level. The survey activities have also engaged with the DINAS extension staff who are embedded in small communities and responsible for adoption of new technology. This information is important in better focusing the research activities within communities and fundamental in identifying key production constraints for farmers. Furthermore, the survey profiled the age, educational background, learning preferences, farm sizes, land tenure, crop rotations and experience of shallot farmers in the key project areas (Brebes, Cirebon, Nganjuk and Bantul) enabling the development of effective engagement and extension strategies for project findings.

8.3.1 Economic impacts

The completion of the literature review has highlighted the key economic constraints to allium production in Indonesia, including geographical and economic constraints. The review also outlines the substantial importation of garlic in Indonesia at about 415,000 tonnes per annum, representing close to half domestic Indonesian shallot production. The document highlights the constraints to increasing allium (shallot and garlic) productivity. This review can be used to support government policy to increase shallot and garlic production and it is anticipated the review will be formally published.

A comprehensive evaluation of production, marketing practices and seed supply systems in shallot cropping has been undertaken to underpin knowledge on key limitations to shallot production and profitability and to understand the factors that impact on the shallot value chain. This information is important in better understanding key cropping costs and the opportunities for the development of a clean shallot seed-bulb system and the productivity gains associated with this system and how the system would function.

Understanding the virus diversity affecting chilli and shallot in Indonesia will assist in better resource allocation for disease resistance breeding. It will also improve effectiveness of disease management for local growers through better selection of control options and thus reduce production costs.

The project has established some virus free shallot material in tissue culture, which is the most important step for identifying the financial impact of viruses on shallot crop productivity and the potential for economic gains through improved shallot seed. Future goals outside of the scope of this project will be to use virus free garlic plants to undertake trials to understand impact from virus, understand vector transmission and rates of reinfection necessary in developing effective clean seed-bulb schemes.

The socioeconomic studies on shallot supply chains shows that the development of high yielding varieties and the supply of quality affordable seed, as opposed to relying on farmers-own seed, is required to improve profitability and sustainability. The project has worked closely with farmers in identifying risk issues in shallot-chilli production and has identified that diversification of farming is required. Shallot is considered a high risk crop, which is prone to production losses and large price fluctuations. This seriously impacts on farmer practices where excessive chemical application on a calendar basis is a key management practice adopted by farmers and is not objectively based.

The nutrient budgeting activity highlighted that most farmers apply almost twice as much fertiliser as is required by shallot crops and for some nutrients the rates are five times crop uptake requirements. Since fertiliser represents the single greatest cost for shallot farmers, substantial savings in fertiliser can be made.

Figure 8.1. the new subtropical garlic cultivar Kenlarge released to industry under the project.

In Australia the reselection of improved garlic lines has resulted in the release of an improved cultivar (cv. Kenlarge) which was selected from the original Glenlarge cultivar. High quality seed of both cultivars has been released and provided to industry under the project. Furthermore, the project has improved nutrient management and general agronomy in garlic, which over the course of the project delivered a threefold yield improvement; from 6.3 t ha^{-1} in 2013 to 17.6 t ha^{-1} in 2018. The project has also sourced commercial quantities of TSS cultivars Sanren and Lokananta from Indonesia and assisted a large onion grower in growing tropical shallots, which were supplied to the Sydney markets at a premium price.

8.3.2 Social impacts

Improvement in the management of major diseases affecting chilli and shallot in Indonesia will improve farm productivity, which has a flow on affect to the broader community in relation to employment, supply of quality product and reduction in exposure to agricultural chemicals. The issue of excessive chemical application and a lack of knowledge on chemical efficacy is a key issue identified in the benchmarking study and the occupational health risks of farming is a key social impact. The projects ToT program addresses occupational health.

8.3.3 Environmental impacts

The training in nutrient budgeting has allowed the Indonesian team to conduct field assessments of nutrient use efficiency (particularly nitrogen), which assessed the proportion of N that is lost from shallot farming systems. The completion of the nutrient budgeting in shallot highlighted that over application of N fertilisers is a key issue and rates of N loss to the environment are likely to be very high. Presentations of this data were made to farmers in October 2014 and provided a basis for farmers reducing their N inputs.

The data from the benchmarking study of shallot farmer practices also highlights very high pesticide application where about 28 applications are made in a 60 day cropping cycle.

Improved management of key diseases of chilli and shallot in Indonesia will have positive environmental impacts through potential reductions in pesticide and fungicide usage.

8.4 Communication and dissemination activities

Training in nutrient budgeting for shallot and chilli was conducted by Dr Stephen Harper and Professor Neal Menzies with staff from IVEGRI, UGM and the Dinas. The training was conducted on 11 April at Nganjuk and 16 April at Cirebon. A copy of the protocol was forwarded to the staff prior to the training and reviewed and amended. The training was conducted as a field demonstration at both sites by going through the procedure.

Training in the molecular detection and identification of viruses affecting alliums was conducted. All participants successfully completed all aspects of the diagnostic assays, including total nucleic acid extraction, cDNA synthesis, PCR and dot blot development. From this, the project team has been able to survey farmers crops and advise on the presence of viral pathogens in crops.

In Indonesia a bench-marking of shallot farmer practices has been conducted by directly interviewing farmers in the Key regions of Cirebon, Brebes, Nganjuk and Bantul. More than 120 farmers across these regions have been interviewed to evaluate demographic agronomic and marketing/supply chain issues and a further 60 traders were interviewed to evaluate supply chain issues. This has resulted in considerable information exchange between the research program and end-users.

In the Australian component, personal invitations were extended to garlic and allium farmers in each season from 2013 to 2018 and were attended by up to 40 farmers each year. These farmers were able to inspect research trials at the DAF Gatton Research Station. One of the most important garlic production practice changes that has been initiated as a result of the project is farmer selection of higher quality seed-garlic. Traditionally, there has been a trend for farmers to market their best quality bulbs and keep the poorer (essentially smaller) material. We have advocated and demonstrated in field trials the negative impact that this has on garlic yields. Dr Stephen Harper presented research findings on improving garlic productivity at the Australian Garlic Growers Association annual meeting in Albury, July 2018. Professor John Thomas also presented research findings on virus impacts on garlic productivity at the same conference.

Project staff, Dr John Thomas and Denis Persley, visited Indonesia (Java) from 23 March to April 3, 2014. The primary purpose of the visit was to undertake a survey of virus diseases in chilli in the Yogyakarta, Cirebon and Brebes regions. The surveys were done in collaboration with the Indonesian project team members from UGM, IVEGRI and IPB and Dr Laurence Kenyon and Dr Wen-Shi Tsai from AVRDC-The World Vegetable Centre, Taiwan.

Seven of the project team members attended the 11th Australasian Plant Virology Workshop held immediately prior to and in conjunction with the IHC2014. During the timeframe of the IHC2014, Sari Nurulita (IPB) completed allium virology training using advanced PCR techniques at the DAF virology laboratories. She subsequently trained other Indonesian scientists in the use of new molecular techniques for identifying allium viruses developed under the project. As part of the congress the project leader arranged a four day bus tour for Indonesian vegetable researchers to evaluate vegetable production systems in South East Queensland, including Bundaberg and the Lockyer Valley. The group was provided a tour of the research facilities at the DAF Gatton Research Station and the Gatton campus of the University of Queensland. The team visited medium and large scale farmers growing a wide range of products including, onions, bunching shallots, cauliflower, lettuce and broccoli as well as the largest Australian chilli producer and intensive protected cropping production systems.

9 Conclusions and recommendations

9.1 Conclusions

The literature review and survey results highlighted several key issues in the shallot-chilli production systems. There is substantial annual growth in shallot production but average yields (9.6 t ha^{-1}) are substantially less than the achievable yield (about half the maximum achievable yield (~20 t ha^{-1})) The survey highlighted that greatly excessive rates of fertiliser and pesticide are applied in shallot production representing a serious impact from an economic cost, health and safety and environmental perspective. The average application of N fertiliser was about 210 kg N ha^{-1} whilst whole crop uptake was only 60 kg N ha^{-1}. Cocktails of up to five pesticides are sprayed on shallot at 2-3 intervals over a 55 day cropping cycle. The survey highlighted a general lack of farmer knowledge of issues around integrated pest management, modes of pesticide action, effective rates, target species, and product safety. Farmers identified that soil fertility and pest and disease management were the two most significant issues for shallot production.

Surveys of disease incidence in shallot and chilli crops across Indonesia highlighted the significance of viruses and fusarium in shallot and PYLCV in chilli. Shallot is predominantly a vegetatively propagated crop in Indonesia but only limited research had been conducted on the impact of viruses on shallot productivity. The survey highlights an extremely high incidence of OYDV, SLV, GarCLV and SYSV in shallot at all sites surveyed across the breadth of Indonesia. Notwithstanding, the survey of farmer perceptions highlighted that viruses were of the lowest priority issue in shallot production. This is likely to relate to the fact that virus infection in shallot simply produces generally less thrifty plants but not striking visual symptoms that are associated with other problems (eg. fusarium and spodoptera). The project findings also identified that shallot yields are greatly impacted by latent virus infections.

In chilli, PYLCV is an endemic pathogen across all of Indonesia. In many surveyed fields infection levels were 100%. The research on genetic resistance to PYLCV shows promise but the current best lines do not express uniform precluding the short-term development of a genetic marker for resistance. The development of genetic resistance combined with other management practices including border cropping strategies, protected seedling production, culling infected plants and shorter season production are important.

A validation of nutrient management practices in shallot production through nutrient budgeting identified greatly excessive application on N fertiliser in Indonesia and represents a considerable environmental risk, though loss pathways remain undefined.

In Australia productivity of garlic in research trials increased from about 4-5 tonnes to about 17 tonnes over the course of the project. Key strategies of seed selection and optimised fertiliser application have delivered these gains and these practises would translate well into Indonesian shallot and garlic production.

9.2 Recommendations

Excessive application of mineral fertiliser and chemicals are serious issues from an environmental, health and safety and economic perspective. Research is required to identify loss pathways and quantify the losses. This needs to be considered in the context of the Surjan system that rotates between double-cropped paddy rice and double cropped shallot raised beds so as to understand the fluxes of nutrient in the overall system. A concerted effort should be undertaken to improve farmer understanding of pests and pesticides since there is clear evidence of misidentification of key pests and a failure to understand the fundamental biology of the target pests and diseases. Furthermore, trialling of new lower toxicity and targeted chemistries should be considered along with improved application technology. This could potentially include remote drone application to improve efficacy and lower the exposure risk of poor farm workers. The development of genetic resistance to PYLCV in chilli germplasm is still identified as a fundamental component of a strategy for managing PYLCV. However, further screening is required aimed at identifying simpler single gene resistance along with improved understanding of vector-virus interaction and vector management is critical.

Given the predominance of bulb propagation in shallot cropping, soil pathogens and viruses are serious problems that can be remediated by the use of true shallot seed. Further research directed to lowering TSS seedling production costs, increasing TSS productivity, increasing the land efficiency factor and reducing the weight or storage loss of TSS bulbs is required. The collaboration between the business sector and government agricultural agencies can greatly improve seed quality and shallot productivity as well as providing new commercial business opportunities for the production of high quality seed.

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10.2 List of publications produced by project

- Detection of Shallots Viruses from Bulb by Dot Immuno-binding Assay. Wulandari AW, Hidayat SH, Sobir. Jurnal Hortikultur (submitted).
- Detection of Major Viruses on Shallots and Garlic from West Java and Central Java. Kadwati, Hidayat SH. Jurnal Fitopatologi Indonesia (submitted).

Poster presentation:

- Hikmah Prima, Siti Subandiyah, Arif Wibowo, Endang Sulistyaningsih, John Thomas,, and Stephen Harper 2014 Shallot Disease Management For Maintaining Bulb Production
- Siti Subandiyah, Alfu Laila, Endang Sulistyaningsih, Arif Wibowo, Yenni Kusandriani, Neni Gunaeni, Witono Adiyoga, John Thomas, and Stephen Harper 2014 Diversity Of Indonesian Shallot Based On Rapd Analysis
- Wibowo, A, Lestiyani, A, Perdhana, K. A, Subandiyah, S, Ito, S, Gambley, C, and Harper, S 2014 Identification of Fusarium sp. The Causal Agent of Basal Root Rot Disease and Its Effect on 4 Shallot Cultivars Grown in Dry Season
- Siti Subandiyah and Arif Wibowo attended the 7th International Symposium on Edible Alliaceae (ISEA2015) in Nigde Turkey 21-25 May 2015 and 2 oral presentations were delivered:
- Sri Hendrastuti Hidayat participated in International Horticulture Conference, Brisbane, Australia (2014). A poster presentation was delivered entitled: Leaf surface characters related to resistance against pepper yellow leaf curl disease.

Oral presentation:

- Novita Pramahsari Putri, Tri Joko, Cherie Gambley, Siti Subandiyah, Yuichi Takikawa, Endang Sulistyninghsih, Arif Wibowo, and Stephen Harper. 2015. Characterization of Soft Rot Bacteria of Shallot (Allium cepa L. aggregatum group)
- Eni Kaeni, Siti Subandiyah, Toekidjo, Endang Sulistyaningsih, and Arif Wibowo. 2015. Response of four shallot (Allium cepa L. aggregatum group) cultivars to moller disease (Fusarium spp) after bulb treatment.
- Siti Subandiyah also gave an oral presentation at Kasetsart University under the program of The 7th International Kasetsart University Science and Technology Annual Research Symposium (I-KUSTARS) 28-19 May 2015:

- Siti Subandiyah, Arif Wibowo, Tri Joko and Sedyo Hartono. 2015. Shallot Diseases in Indonesia.
- Sulistyaningsih, E, Subandiyah, S, Wibowo, A, Heskiel, A, Harper, S, and Thomas, J. 2014. Possibility Of True Seed Shallot Production In Low Land Areas In Indonesia.
- Sri Hendrastuti Hidayat and Sari Nurulita participated in Australian Plant Virology Workshop, Brisbane, Australia (2014). Two oral presentations was delivered :
- "Detection of viruses on shallot from West Java and Central Java" by Sari Nurulita
- "Geminivirus Associated with Weed Species Found in Chilli Pepper Field in Java, Indonesia" by Sri Hendrastuti Hidayat

Appendixes

Appendix 1:

BASE LINE SURVEY PROTOCOL
ACIAR Allium

Background

One of the activities in the Hortin Project (Joint R & D of vegetables between Indonesia and the Netherlands) is the implementation of a mini survey for shallot in Brebes, Central Java, involving 5 farmers and 5 traders as respondents. Surveys at the farm level, briefly elaborate (a) production and cost of production, (b) utilization, (c) post-harvest, (c) price, (d) marketing, (e) access to services, and (f) problems and constraints. While at the trader level, surveys summarize (a) volume and sources of supply, (b) supplier/ vendor, (c) buyer, (d) price, (e) transactions, (f) post-harvest, (g) cost and marketing risks, and (h) opportunities and constraints to development. The survey was originally intended as a pre-survey for value chain analysis that its implementation was canceled because of priority setting reasons (resources).

ACIAR Allium-Project – planned baseline survey is much more elaborative and comprehensive. Three key research questions that need answers are: (1) How is the portrait the techno-socio-economic progress/status of the shallot farming? (2) How is the description of shallot value chain? (3) How does the existing shallot seed supply system work? These three questions are essentially different to each other. Thus, all of these questions are impossible to be responded by one-single survey. Hence, the baseline survey will consist of three activities, namely, (1) farm-analysis survey - technical and non-technical aspects of shallot farming, (2) shallot value chain analysis-survey, and (3) shallot seed focus group discussion. The scale or magnitude of each activity will be adjusted to conform to the availability of allocated resources as agreed in the project proposal.

Methodology

Baseline survey was designed as a combination of descriptive survey (portraying present situations/conditions or describing reality at a given point in time) and the exploratory survey (collecting basic information that may help the problem identification and hypothesis formulation). Activities held in Cirebon and Brebes will be coordinated by IVEGRI, while those carried out in Yogyakarta and Nganjuk will be coordinated by UGM.

Data and information collected in these activities are consisted of:

(1) Farm-analysis survey - technical and non-technical aspects of shallot farming

1. Profile of Respondents
2. Vegetable Farming - General
3. Aspects of Shallot Cultivation
    - General
    - Seed
    - Planting
    - Soil Treatment
    - Fertilization
    - Pest and Disease Control
    - Irrigation
    - Harvest
    - Constraints
4. Aspects of Shallot Farming
    - General
    - Seed Cost
    - Fertilizer Costs
    - Pesticide costs
    - Weeding Costs
    - Irrigation Costs
    - Soil Preparation Costs
    - Investment Costs
    - Machinery / Equipment Costs
    - Harvesting Costs
    - Miscellaneous Costs
    - Production and Income
    - Marketing

(2) Shallot value chain analysis-survey

1. Supporting information / Description of activity
2. Specialization (related to economies of scale and risk strategy)
3. Description of trade and flow of products
4. Price formation and marketing costs
5. Price risk
6. Volume and other traded products
7. Suppliers, buyers and transactions
8. Market risk
9. Quality
10. Storage
11. Risk storage
12. Handling, sorting and packing

13. Transportation
14. Liquidity
15. Credit to the buyer
16. Credit from the seller
17. Competition
18. Skills
19. Information
20. Innovation
21. Support services
22. Policy and regulation
23. Constraints and business opportunity

(3) Shallot seed focus group discussion

1. Seed sources
2. Seed renovation
3. Flow / distribution of seeds
4. Cost of seed production
5. Seed price and price determination seed
6. Management of seed quality
7. Evaluation of farmers' seed systems
8. Supports from public services
9. Constraints and opportunities

Respondents for the farm-analysis survey are shallot growers who will be randomly selected. At each study site (Cirebon, Brebes, Yogya and Nganjuk) 30 respondents will be interviewed individually.

Respondents for value chain analysis survey are traders (collectors, large/wholesaler, retailer) of shallot. For each type, 3-5 traders are purposively selected at each study site and those traders will also be interviewed individually.

Participants for the shallot seed focus group discussion will also be selected purposively and consists of farmer-representatives (3-5 persons/location), seed growers-representatives (3-5 persons/location) and officers from regional agricultural office or related public institutions (2 persons/locations).

Data collection and processing

Data collection for the farm analysis and value chain analysis is carried out through surveys - interviews, while collecting data for seed systems assessment is carried out through focus group discussions. A questionnaire (interview instruments) and guiding questions (focus group instruments) are prepared and agreed by all parties (collaborators).

Data processing will be conducted in each institution (IVEGRI and UGM) by using the same method.

Data analysis

Collected data will be analyzed using descriptive statistics (surveys) and content analysis (focus group discussions).

Reporting

The survey and focus group discussions will be reported internally to the members of the project team. Reporting and assessment of the results of these activities are scheduled to be held in March 2013.

Schedule of activities

		Oct	Nov				Dec				Jan 2013			
	Activity		week				week				week			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
1	Preparation of the baseline survey protocol	X												
2	The drafting of questionnaires and guiding questions	X												
3	Protocols and instruments discussions via email	X	X											
4	Preparation of implementation activities - coordination and permits, etc.		X											
5	Implementation of the farm survey			X		X	X		X					
6	Implementation of the value chain survey			X		X	X		X					
7	Implementation of seed systems focus group discussions										X	X	X	

Appendix 2

Protocols for Allium research: ACIAR Project

- ## Investigating the diversity of virus in Australian garlic

- ### Aim

The aim of this study is to determine what viruses are present in Australian grown garlic. The study will involve development of virus-specific assays for virus detection and survey of Australian cropping districts.

Methods

Virus-specific assay development

There are at least 16 different virus species known to infect garlic worldwide. Of these 16 species, eight are allexiviruses, four are potyviruses, two area carlaviruses and a single nepovirus and tospovirus. The virus species are:

- Allexiviruses:
  - Garlic mite-borne filamentous virus (GaMBFV): possibly unpublished, Genbank record
  - **Garlic virus A (GVA)**
  - **Garlic virus B (GVB)**
  - **Garlic virus C (GVC)**
  - **Garlic virus D (GVD)**
  - Garlic virus E (GVE)
  - Garlic virus X (GVX)
  - Shallot mite borne latent virus (aka Shallot virus X; ShMBV and ShVX, respectively)
- Potyviruses:
  - **Leek yellow stripe virus (LYSV)**
  - Leek yellow stripe virus – garlic yellow streak strain
  - **Onion yellow dwarf virus (OYDV)**
  - Shallot yellow stripe virus (ShYSV)
- Carlaviruses:
  - **Garlic common latent virus (GCLV)**
  - Shallot latent virus (aka Garlic latent virus; ShLV and GaLV, respectively)
- Nepovirus: Tomato black ring virus (TBRV)
- Tospovirus: Iris yellow spot virus (IYSV)

The species highlighted in bold are known to be present in garlic in Australia. To detect these viruses and any potential newly emerged viruses within these virus genera, genera-specific degenerate primers were used in RT-PCR. For all virus species other than IYSV, the oligodT reverse primer Poty1, will be used to generate cDNA and to amplify the cDNA with the appropriate genera-specific degenerate forward primer. The forward primers used in PCR were pGV-3t for allexiviruses, pCarl for carlaviruses, nepoviruses and U341 for potyviruses.

Symptomatic garlic samples were selected from commercial fields and tested by RT-PCR for these viruses. Leaf tissue was extracted using the Qiagen Biosprint RNA extraction kit as per manufacturer's instructions. Subsequent amplicons were cloned and multiple clones evaluated by RFLP using the restriction endonucleases as per manufacturer's instructions. Unique RFLP patterns were identified and their corresponding clones sequenced in both directions. This process was repeated with further samples until no further unique patterns were identified.

To develop specific assays for routine indexing of survey samples, reference clones for each virus were used to generate specific probes for use in dot blot assays. The specific virus assays used degenerate genera-specific RT-PCR to amplify genome fragments of virus populations within samples then virus-specific probes to identify individual virus species within that population. This was done by blotting 1 µL aliquots of each RT-PCR onto a Hybond-N+ membrane, with a separate

membrane used for each virus target. The membranes were then exposed to the virus-specific probes and developed using a colorimetric system.

Investigating the distribution of virus in Australian garlic

Aim

The aim of this study is to determine if there are differences in distribution of allium-infecting viruses within garlic bulbs. If differences are observed data will be correlated with bulb and clove sizes to look for potential associations between these sizes and presence or absence of virus. Additionally, the presence or absence of virus in bulbils will be investigated. A hopeful outcome from the experiments is detection of virus-free material.

Methods

Bulbs and bulbils from selected plants will be propagated using an 18°C night/23°C day temperature cycle in a climate controlled glasshouse. Germinated plantlets will be maintained insect free to prevent cross infection of viruses.

Experiment 1: investigating virus incidence in garlic bulbs

Cloves from bulbs collected from (1) plants with good agronomic presentation, (2) plants with poor agronomic presentation and (3) random plants will be planted individually then rated for symptoms and indexed for viruses one-month after planting. Each bulb will be weighed and then each clove within the bulb weighed prior to planting. At least 10 bulbs each from variables (1), (2) and (3) will be evaluated and are likely to provide about 10 cloves per bulb. The bulb material used in this experiment is all Glen Large.

Experiment 2: investigating virus incidence in garlic bulbils

Bulbils collected from (1) Glen large plants with good agronomic presentation, (2) Glen large plants with poor agronomic presentation and (3) from red garlic will be planted. In addition, the bulbs from these plants will be planted. For variables (1) and (2) these bulbs will be those used in experiment 1. At least 20 bulbils per variable will be evaluated.

Virus indexing will involve testing each plantlet individually by RT-PCR as described above using the degenerate genera-specific assays. If required individual virus species will be differentiated using virus-specific probes in a dot blot assay. Any plantlets identified as virus-free will be further propagated by tissue culture.

Investigating the virus status of Indonesian shallot

Aim

The aim of this study is to answer the following questions:

1. What is the diversity of viruses affecting Indonesian shallot crops?
2. What virus is the most prevalent throughout the cropping district(s) of Indonesia?
3. What virus is having the most economic impact?

4. Are there virus-free areas available for seed production?

There are two major shallot production areas requiring survey. The first is in East/Central Java and includes the districts around Nganjuk and Yogyakarta. The second is in West/Central Java and include the districts around Brebes and Cirebon. The majority of the surveys to be conducted by the Indonesians, with staff from the University of Gadjah Mada (UGM) completing the East/Central Java and those from IVEGRI doing the West/Central Java surveys.

- ## Method

The survey strategy will depend largely upon the expected incidence of virus disease within crops. If virus disease is quite low, visual inspections will provide statistically relevant data at very low cost. By contrast, if disease is very high, samples will need to be molecularly indexed.

The number of plants to be inspected and/or sampled is determined by the expected level of each virus if present and the desired confidence of the results. For example, if the virus is expected at an incidence of 1% or more, then inspection/sampling of 300 plants will provide a 95% confidence of detecting that virus. These numbers are predicted by the statistical model described by Cannon and Roe (1982). A summary of their model is shown in Table 1.

P (%)	**Population size**							
	50	**75**	**100**	**300**	**500**	**1000**	**5000**	**10 000**
a) 90% probability of detection at incidences from 0.5 to 10%								
0.5	50	75	100	271	342	369	439	449
1	45	68	91	161	184	205	224	227
5	27	33	37	42	43	44	45	45
10	18	18	20	22	22	22	22	22
b) 95% probability of detection at incidences from 0.5 to 10%								
0.5	50	72	100	286	388	450	564	581
1	48	72	96	189	225	258	290	294
5	31	39	45	54	56	57	69	59
10	22	23	25	28	28	29	29	29
c) 99% probability of detection at incidences from 0.5 to 10%								
0.5	50	75	100	297	450	601	840	878
1	50	75	99	235	300	368	438	448
5	39	51	59	78	83	86	89	90
10	29	32	36	41	42	43	44	44

If crops are relatively healthy and virus symptoms readily detectable, then visual inspections of at least 300 plants within a crop is recommended and should include two transects through the crop and two edges/corners. Given the high planting density of shallot the actual numbers of plants inspected is likely to be much greater than this thus easily meeting the statistical requirement. If virus presence is detected through presence of symptomatic plants then selected plants should be sampled and tested by molecular indexing. It is recommended to sample at least 10 plants per symptom type at least at the first site. At subsequent sites if the same symptom types are present then sampling of 2-3 plants per symptom type would be sufficient.

If there is a high incidence of virus disease and/or symptoms are not readily detectable because of the presence of other diseases or nutritional disorders than a random sampling strategy will be needed followed by molecular indexing. The number of samples collected is dependent on the stringency of the survey. For example, collection of 300 samples would provide very good confidence of virus detection if present. However, if it is only necessary to know if viruses are present when they are at an incidence of 10% or more then only 30 samples would be required for a 95% confidence.

Given the crops are vegetatively propagated, there is no strategic advantage in surveying any particular sections of the crop relative to others. For example, with insect-transmitted viruses there is an advantage in surveying edge rows compared to the centre of the crop. With vegetatively propagated crops the virus incidence within the field is expected to be random. Edge effects could still occur if there is also movement into the crop of insect-vectored viruses from surrounding crops or fields (e.g potyviruses and tospoviruses). The proposed survey strategy is to inspect some plants along the edges or corners of the block in addition to transects through the block.

• Investigating the disease status of Indonesian shallot other than viruses

• Aim

The aim of this study is to answer the following questions:

1. What are the major non-viral diseases affecting Indonesian shallot crops?
2. What disease is the most prevalent throughout the cropping district(s) of Indonesia?
3. What disease is having the most economic impact?

There are two major shallot production areas requiring survey. The first is in Central Java and includes the districts around Nganjuk and Yogyakarta. The second is in West Java and include the districts around Brebes and Chrebin. The majority of the surveys to be conducted by the Indonesians, with staff from the UGM completing the Central Java and those from IVEGRI doing the West Java surveys.

• Method

Crops will be surveyed for diseases other than those caused by viruses, most notably is fusarium. Similar numbers and logic apply to these surveys as does to the virus surveys. To survey for fusarium by visual inspection the symptoms will need to be specific. If other diseases are present and induce similar symptoms to fusarium then visual examinations may become meaningless. A recommended strategy is to follow closely that above for viruses and collect at least 30 symptomatic plants from each crop for isolations, provided the incidence of the disease is estimated to be around 10% or more.

If multiple races of fusarium are present in Indonesia, further characterisation of selected isolates through differential germplasm lines will be required to evaluate if any particular race is dominant. Alternatively molecular assays may exist to do this.

What other diseases do we need to survey for? Anthracnose and Alternaria area likely to exist but if not causing yield problems are probably not very important.

Questions about fusarium?

1. what identifications are done to date? geographical spread of isolates, typing methods, molecular sequencing etc
2. are there races of fusarium known to exist in Indonesia or elsewhere?
3. what other diseases in Indonesia may cause similar symptoms in the field and thus complicate surveys?
4. is it a field problem with fusarium or does storage fusarium species cause problems as well?

Appendix 3

Evaluating crop nutrient uptake and fertiliser use efficiency

This protocol is for the assessment of shallot and chilli crop nutrient removal and fertiliser inputs and how to conduct field sampling for this. From this the fertiliser use efficiency can be determined.

The following information needs to be determined to calculate the crop nitrogen uptake and fertiliser use efficiency:

- What is the crop yield (tonne/ha) including the yield of residues.
- How much nitrogen did your crop remove in harvested product.
- How much nitrogen was returned to the soil system as crop residues.
- How much fertiliser nitrogen was applied (kg/ha).

The following guideline outlines how to collect and calculate this information, how to sample and finally how to calculate your nitrogen use efficiency.

• *Step 1. Determine the yield of the harvested crop product and the yield of crop residues.*

You need to calculate the yield of the harvested product and crop residues of shallots and chillies since the amount of N removed by your crop depends on yield.

Determining shallot crop yield.

For shallot select 4 subplots for sampling within one farmer-field and use the following procedure to measure yield for each of the four samples within the crop. The subplots are essentially replicates that will be averaged to give a more accurate estimate of nutrient removal and fertiliser use efficiency. Make sure the subplots are representative of the field. For example, if one area of the field has severe fusarium then avoid sampling from this spot. However, if for example, fusarium was widespread select spots that are broadly representative of the field.

The calculations require the measurement of both the harvested shallot bulb yield as well as the yield of the crop residues (shallot foliage/tops).

Sample and weigh the crop
At crop maturity measure a 3 m length section of a bed representative of how the field looks. For this 3 m section harvest the shallots including the tops (foliage) into a harvest bin. Weigh the whole plant sample and record the fresh weight. Then separate the tops from the shallot bulbs into one pile of shallot bulbs and one pile of the plant tops. Weigh the shallot bulbs and the plant tops and record the fresh weight of each.

The fresh weights of both the shallot bulbs and the crop residue sample are used to calculate the yield of each of these components. To calculate shallot bulb and residue yield

you will first need to also know the bed width and the calculated harvested plot area. These are achieved by the following:-

Calculate bed width:- Accurately measure the width of the beds. Use a tape measure to measure the distance across 5 beds and divide the measured distance by the number of beds to give the average bed width.

Calculate harvested plot area:- The harvested plot area (m^2) is calculated by the plot length (m) x bed-width (m). The bed width will be taken from the centre of the surjen furrow to furrow.

The fresh yield is calculated by the following:-

Calculate fresh yield - To calculate fresh yield use the following formula.

Fresh Yield (tonne/ha) = 10,000 (m^2/ha) / harvested plot area (m^2) x weight for harvested plot / (1000 kg/tonne).

See Example 1 for this calculation

Example 1:- Farmer A sampled a 3 m long plot in his shallot crop just before harvest. The total plant (shallot bulb and foliage) weight for the plot was 13.18 kg. The weight of the shallot bulbs was 7.83 kg and the weight of the foliage crop residues was 5.35 kg. The measured distance across five beds was 7.6m giving an average bed width of 1.52m (furrow to furrow).

Harvested plot area = plot length (m) x bed-width (m).

3.0m x 1.52m = 4.56 m^2

Fresh Yield (tonne/ha) = 10,000 (m^2/ha) / harvested plot area (m^2) x weight for harvested plot / (1000 kg/tonne).

Shallot bulb yield (tonne/ha) = 10,000 (m^2/ha)/ 4.56 m^2 x 7.83 kg / 1000 (kg/tonne).

= 17.17 tonne/ha

Taking subsamples for analysis

Take sub-samples of about 0.8-1.0 kg of the harvested shallot bulbs and foliage residues from the plot samples. Accurately measure and record the weight of these samples using a balance that is accurate for small samples (to one decimal place). Place each sample in a paper bag (labelled with farmer or sample name, plant part (foliage or bulb), date, replicate and other relevant detail) and take them back to your laboratory or research station to dry them. Now go to the section below "***General Shallot and Chilli sample Information".***

Collecting samples and calculating harvested product and crop residue yield for crops with multiple harvests such a Chilli.

The sampling of crops with multiple harvests on the same plants (e.g. chillies, tomatoes, capsicums, cucurbits etc.) is a little more complicated. This is because it is more complex to measure yield since repeated sampling is required as the crop matures. If you have difficulties undertaking the following procedure seek advice.

There are 2 options you can use to work out the yield of crops with multiple harvests. You need to look at each of these and choose which is best for you.

Chilli Market yield method. The **easiest** way to measure yield for these crops is to use harvest records and the weight recorded at the market site for each load delivered. Add the loads to give a total weight and divide the total harvested tonnage by the area of the block that was harvested. **However, the farmer must keep very good (accurate) records to make sure you can accurately calculate the tonnage recorded at each harvest and be sure of the block area.** If done correctly, this will give a very accurate yield. For this calculation refer to Example 2 below.

Example 2. A chilli grower had 3 harvests on a block of chillies and the tonnage recorded at each harvest from the block was 0.26, 0.31, and 0.17 tonne giving a total of 0.74 tonne. The total area of the farmed chilli block was 0.1 ha.
Chilli fruit Yield through market (t/ha) = 0.74 tonne / 0.1 ha
= 7.4 tonne/ha

Chilli Tagged or marked plants method. The other way to measure yield is to tag 20 random plants in the field with tape (avoiding obviously unhealthy plants) and harvest these exact same plants until final harvest. Record the weight of product at each harvest. **However, you must make sure that these plants are not accidently harvested by field-workers during the normal commercial crop harvest.** These harvest weights can then be used to calculate the yield of product using the following formula:

> **Chilli fruit yield (t/ha)** = sample weight (kg) / number of plants sampled x plant population (plants per ha) / 1000 (kg/tonne). An example is shown below (Example 3.).

Example 3. A chilli farmer has tagged 20 plants and hand harvested these at 3 different times, each at about the time the rest of the field was harvested. The total weight of product (from the 15 plants) at each harvest was 0.56, 1.20 and 0.73 kg (for harvests 1, 2 and 3) giving a total over the 20 plants of 2.49 kg. The total plant population was 40,000 plants per ha.

Yield (t/ha) = sample weight (kg) / number of plants x plant population (plants per ha) / 1000 (kg/tonne).

Chilli Fruit Yield (t/ha) = 2.49 kg / 15 x 40,000 / 1000
= 6.64 tonne/ha

Measuring Chilli crop residue yield:- Regardless of whether you use the market yield method or tagged plants method you will also need to sample the chilli crop residues (the chilli bush) at the final harvest to calculate the crop residue yield and also the N contained in them. To do this, after the final harvest collect 15 typical (healthy) plants (excluding the roots) and weigh these. If you used the tagged plant method you can harvest your tagged plants. Calculate the yield of the chilli crop residues using the following:

Chilli Crop residue Yield (t/ha) = sample weight (kg) / no of plants x plant population (plants per ha) / 1000 (kg/tonne).

An example is shown below (Example 4.).

Example 4. The chilli crop was sampled at final harvest for crop residues. Fifteen (15) Plants were harvested and these had a combined foliage and stem weight of 2.67 kg. The total plant population was 40, 000 plants per ha.

Chilli Crop Residue Yield (t/ha) = sample weight / number of plants sampled x plant population (plants/ha) 1000 (kg/tonne).

Chilli crop Residue yield (t/ha) = 2.67 kg / 15 plants x 40,000 plants/ha / 1000 kg/tonne
= 7.12 tonne/ha

Taking Chilli samples for analysis

Fruit sample. At the final harvest collect a sample of approximately 0.8-1 kg sample of the chilli fruit. Weigh each sample precisely, using a balance with one decimal place accuracy and record the exact weight of the sampled fruit. The harvested fruit sample can be obtained from the growers harvested crop for market or from the plants in the field. After weighing the sample and recording the weight place the samples in a paper bag and take them back to your laboratory or research station to dry them.

Crop residue sample. At harvest retain a representative sample of about 0.8-1 kg of the chilli crop plant residues (with a portion of leaf and stem from the 15 plants). Weigh each sample precisely, using a balance with one decimal place accuracy and record the exact weight of the crop residue sample. Place these in a paper bag labelled with the sample details (farmer, crop, sample type (fruit or foliage), date, sample fresh weight and replicate (if required)) and take them back to your laboratory or research station to dry them.

General Shallot and Chilli sample Information

Dehydrating samples. Take the fresh shallot bulb and foliage or chilli fruit and foliage sub-samples back to your laboratory or research station and immediately place them in a dehydrating oven at about 75ºC for 3 days. If required cut the samples into smaller pieces to allow more rapid drying. After this, take the samples from the oven and immediately weigh the sample and record the dry weight. If this is not done immediately the samples will absorb moisture, particularly when it is humid and this will give inaccurate results. If the samples do take up moisture before being weighed they will require redrying prior to weighing them. Retain the samples so they can be analysed for nitrogen or other nutrient content in your laboratory.

Calculating the dry matter content of the samples
It is necessary to calculate the dry matter content of your samples because the Nitrogen (and other nutrient analyses) will be conducted on a dry sample and not the fresh sample for which you have the yields. The dry matter of the samples is calculated by dividing the sample dry weight by the sample fresh weight and multiplying by 100, to give a percentage. For example

Example 5. A shallot bulb subsample was taken from a field (eg as in example 1). The subsample was weighed and was exactly 874.3 g. The sample was dehydrated and weighed and was exactly 142.5 g. The dry matter content is calculated by

Dry matter (DM%) = sample Dry Weight / Sample Fresh Weight x 100
= 142.5 g / 874.3 g x 100
= 16.3%

A shallot foliage subsample was taken from a field (eg as in example 1). The subsample was weighed and was exactly 923.2 g. The sample was dehydrated and weighed and was exactly 97.6 g. The dry matter content for the foliage is calculated by

Dry matter (DM%) = sample Dry Weight / Sample Fresh Weight x 100

= 97.6 g / 923.2 g x 100

= 10.6%

Laboratory analysis for N or other nutrients

Send your dried plant samples to the laboratory for an N analysis or other nutrient analysis to be conducted.

Calculating Shallot bulb or chilli fruit N removal and crop residue N.

The above procedure has allowed you to assemble the information required to calculate the nitrogen removed in the harvested shallots or chillies and the amount taken up in the crop residue biomass. These pieces of information needed to calculate the N removal include:-

- yield (tonne/ha) of harvested product and crop residues
- The dry matter content (%) of harvested product and crop residues
- The N content (N%) of harvested product and crop residues

The N uptake and removal per ha in the crop and its residues can be calculated using the following:-

N uptake/removal (kg/ha) = yield (tonne/ha) x dry matter (%) /100 x sample N content (N%) x 1000 (kg per tonne).

An example of a calculation using the shallot bulb examples for yield (Example 1) and bulb dry matter (Example 6).

Example 6. The yield results for shallot bulbs and foliage (Example 1) and the matching dry matter contents (Example 5) will be used as the basis for the N uptake and removal calculations. The yield and DM% for the shallot bulbs were 17.17 tonne/ha and of 16.3% respectively. The yield and DM% for the foliage residues were 11.73 tonne/ha and of 10.6% respectively. The N content measured by the laboratory were 1.9% for the shallot bulbs and 2.4% for the foliage.

N uptake/removal (kg/ha) = yield (tonne/ha) x dry matter (%) /100 x sample N content (N%) x 1000 (kg per tonne).

The shallot bulb N removed (kg/ha) = 17.17 x 16.3 / 100 x 1.9/100 *1000

= 53.2 kg N/ha

The N in shallot plant residues (kg/ha) = 11.73 x 10.6 / 100 x 2.4 / 100 *1000

= 29.8 kg N/ha

- ## *Step 3. How much nitrogen did you apply*

All fertilisers contain different percentages of nutrients and the amount of N in these has to be calculated to compare this with what the crop has removed.

Obtain a record from the farmer of the amount and type of fertiliser that has been applied to the crop including the base fertiliser and any in-crop (sidedress) applications as well as manure or compost applications.

- Calculate the total kilograms of nitrogen applied per hectare. Do this by multiplying the amount of fertiliser applied (kg/ha) by the percentage of N in the fertiliser (you can usually get this from the fertiliser packaging).
- Then add up the N applied in each fertiliser application.

  Nitrogen (kg/ha) = % N in fertiliser/100 x rate of fertiliser applied per ha

Example 7. A typical example might be

Fertiliser	Amount of N in fertiliser	Amount of fertiliser applied (kg per ha)	Calculation of N applied	Amount of N applied in fertiliser (kg/ha)
Nitrophoska	12.0%	500	=12.0/100 x 500	60.0
Calcium Nitrate	17.1%	40	=17.1/100 x 40	6.8
Potassium Nitrate	13.8%	80	=13.8/100 x 80	11.0
Ammonium	21.2%	100	=21.2/100 x 100	

Step 3. Calculating your fertiliser use efficiency

To calculate your fertiliser use efficiency you need the total applied nitrogen values from Step 1 and the N removal values from Step 2. Your fertiliser use efficiency can be calculated by dividing the amount used in the plant by the amount of nitrogen you applied.

This will allow you to see how well your applied N matches crop removal and how much loss may be in your system. You may use this data to fine tune your fertiliser program or look at ways to minimise possible losses.

Example 8. In the prvious example for shallot we showed a total of Example 1 we calculated that 99.1 kg of N was applied to a shallot crop and we calculated that 83.0 kg was taken up/required to grow the whole crop including the harvested shallot bulbs and residues of which 29.8 kg of N per ha was in residues.

The Nitrogen use efficiency = nutrient taken up / nutrient applied x 100

Nitrogen use efficiency = 83.0 / 99.1 x 100

Please note: This is based on a partial nitrogen balance only and does not take into account all sources and losses of nitrogen to and from your system and root sytem nutrient requirements. Only fertiliser applied N and crop removal figures are considered.

- ### *Interpreting your nitrogen use efficiency values*

Ideally you should be aiming for a nitrogen use efficiency value as close to 100% as possible. This would suggest that the fertiliser you are applying matches plant use and removal in harvested product. In practice this is difficult to achieve as some N loss is unavoidable.

Nitrogen use efficiency value	**What does this mean?**
Less than 100%	You are applying more nitrogen than your crop is using and removing
About 100%	You are applying nitrogen at rates that roughly matches crop use and removal
Greater than 100%	You are applying less nitrogen than your crop is using and removing. Other sources of nitrogen are making up the shortfall.

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Understand your crop's harvest index

Vegetable crops need nutrients to produce the harvested part of the plant but also to grow the other unharvested plant parts that end up as crop residues. Crop residues returned to the soil will have some of the fertiliser used by the plant to grow the crop. This amount depends largely on the *crop harvest index*. Understanding your crops harvest index can give you an idea of how much nitrogen may be returned to the soil system in residues. The harvest index refers to how much the harvested product is as a proportion (%) of the total plant (crop) biomass. In vegetable crops the proportion of a plant that is harvested can vary considerably. The harvest index can indicate how much nitrogen is removed by a crop.

High harvest index (80-90%) Examples include lettuce, potato and cabbage where almost the whole plant is harvested and sold except for the roots and a very small amount of foliage.	

Intermediate harvest index (50-70%) Examples include Chilli, tomato, capsicum and cucurbits. These crops have a balance between how much of the plant is harvested and how much is returned to the soil as plant residue.	
Low harvest index (30-40%) Examples are broccoli, sweetcorn and green beans. The amount of product harvested as a proportion of the total plant is low.	

Understanding the harvest index of different crops is important because the nitrogen in the unharvested crop residue becomes available for following crops and should be considered in fertiliser rate decisions.