VG508
Tomato germplasm conservation

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QDPI

Know-how for Horticulture™

VG508

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Final Report

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Tomato germplasm conservation

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Queensland Department of Primary Industries

Final Report of HRDC project VG508 "Tomato germplasm conservation"

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1 Industry Summary

Tomato germplasm is the raw material, the source of new genetic variability, used by plant breeders to develop new cultivars of tomatoes.

The tomato germplasm collections being maintained at QDPI Bowen Research Station and Redlands Research Station were transferred to the -20°C long term seed store at the Australian Tropical Crops Genetic Resource Centre, Biloela. Only 16% of the accessions in the collections had sufficient quantity of viable seed suitable for long-term storage, while 4% of the accessions had less than 50 seeds, and 42% of the accessions had >50% "fresh" seed which is seed that is viable but has some form of dormancy.

A range of experiments were conducted to determine the best method of germinating the "fresh" seed so that these accessions could be regenerated and new good quality seed placed in long-term storage. The best method of promoting germination and growing accessions to regenerate seed was:

- seed scarification under sterile conditions
- grow seeds in culture medium of agar and MS salts in an incubator at controlled temperature and light regimes
- at the 4-leaf stage move seedlings to greenhouse and allow plants to harden off
- transplant seedlings to rich potting mix
- routinely control powdery mildew which is a problem in greenhouses
- pollinate wild tomato species with a mechanical vibrator to increase seed set
- extract seed from fruit using dilute acid solution
- dry seed slowly to 6% seed moisture
- seal seed stocks in laminated aluminium seed packets and place in the -20°C long term seed store where they should remain viable for 50-100 years

Using this method 450 accessions were regenerated during the project. 70% of the accessions now have sufficient quantities of viable seed. Small quantities of seed (50 seeds) are freely available to research clients on request. Passport and evaluation data on all accessions in the germplasm collection are available via the internet (http://www.dpi.qld.gov.au/auspgris/). By using a web browser clients can easily search the database and lodge requests for seed.

The Australian Tropical Crops Genetic Resource Centre will continue to maintain the national tomato germplasm collection and to provide seed to research clients on request. An Australian germplasm collection is a form of national insurance, as global intellectual property laws restrict the exchange of germplasm between countries making it more difficult to re-import germplasm in future years.

2 Technical Summary

Regeneration of the national collection of tomato (*Lycopersicon*) germplasm, now stored at the Australian Tropical Field Crops Genetic Resource Centre, Biloela was required because many accessions had small quantities of seed. However, problems were encountered with "fresh" seed that was not germinating under normal conditions because there was some form of dormancy. There was the distinct possibility of losing much of the genetic variability within the accessions or even losing the whole accession completely.

A number of experiments were conducted to develop a procedure for germinating "fresh" seed. The best method involved scarification of seeds by nicking them with a scalpel under sterile conditions; germination rates increased from 0% to an average of 90%. Other seed treatments including chilling, acid scarification, gibberellic acid, and potassium nitrate made no difference to germination rates. The seeds were germinated on a culture media of agar and MS salts in a environment incubator. Sugar was omitted from the culture media to reduce fungal contamination. The additional of sodium chloride to the MS media inhibited germination rates. Seedlings were transferred to pots at the 4-6 leaf stage and grown to maturity.

This procedure for germinating and growing tomato seedlings was used to regenerate the national tomato germplasm collection which currently consists of 15 species and a total of 1216 accessions. 70% of the accessions now have sufficient quantities of viable seed in long-term storage. The remaining 30% off the accessions will be regenerated over the next three years.

Under another project, a national database system has been establish for all *ex-situ* plant genetic resources in Australia, the Australian Plant Genetic Resources Information System (AusPGRIS), which is available via the internet (http://www.dpi.qld.gov.au/auspgris/). Passport and characterisation information for the tomato germplasm collection has been included in AusPGRIS and clients can search the database and lodge requests for seed.

3 Introduction

3.1 Background

Germplasm collections of fruit and vegetable crops in Australia are currently maintained by various individual projects within State Departments of Agriculture, by private seed companies, and by non-government organisations. Maintenance of the collections is often a low priority and some collections are in danger of being lost. Passport and characterisation records are scanty and often not computerised. There is no national approach to the conservation of fruit and vegetable plant genetic resources.

Throughout the world, plant genetic resources are becoming more difficult to acquire as improved cultivars replace landraces and as G77 countries realise that plant genetic resources are a valuable asset which can be subject to negotiations. Australian horticultural crops originate from overseas countries. Therefore, once germplasm is imported into Australia it is essential that the germplasm be conserved for possible future use instead of assuming it may be possible to reimport the germplasm in the future.

The Australian Network of Plant Genetic Resource Centres was established by SCA in 1982 to conserve plant genetic resources of field crop and forage species. In 1987 two PPC/SCA Working Parties, on Vegetable Germplasm and Fruit & Nut Tree Germplasm, recommended that a national database be established for all horticultural germplasm, but that a national repository for horticulture germplasm was not warranted. No action resulted from these recommendations.

One of the Centres within the Network, the Australian Tropical Crops Genetic Resource Centre operated by QDPI at Biloela Research Station, has the expertise to regenerate and conserve seed of germplasm accessions, and to operate a germplasm database for a wide range of crop species. The opportunity exists for the Centre to extend its mandate to include, (i) maintaining a database on all tropical fruit and vegetable germplasm collections, and (ii) conservation of tropical fruit and vegetable accessions which can be stored as seed.

The benefit of one national approach to the conservation of horticulture germplasm is that each researcher will have access to a comprehensive database on passport and characterisation data as opposed to a very minimal data-set.

3.2 Description of the problem/opportunity

QDPI has the largest collection of tomato (*Lycopersicon*) germplasm within Australia, 340 tomato accessions at Redlands Research Station, and 1300 tomato accessions at Bowen Research Station. Seed at Redlands Research Station is 20 years old and is stored at 5°C, records are non-existent, and the collections may be lost if action is not taken immediately to conserve these accessions. The tomato germplasm at Bowen Research Station is stored at 5°C, and passport & characterisation data on disease resistances and susceptibilities plus remarks on agronomic traits are stored on a computer database. Seed of these tomato accessions is in demand, especially wild species, and the main problem encountered by the tomato breeder is finding time to regenerate 200 accessions per year.

NSW Agriculture at Gosford have a collection of 200 tomato germplasm accessions. Seed is stored at 6°C, there is no database, and the project has difficulty with seed regeneration and maintenance.

The Seed Savers Network and Heritage Seeds, non-governmental organisations composed of volunteers who operate nationally, have approximately 2000 accessions of vegetables in their collections. Both organisations have good contacts with the community and are able to obtain seed of many heritage varieties. However, they have no seed storage facilities and rely on a network of home gardeners throughout Australia to maintain the seed. Their efforts would be complementary to our Genetic Resource Centre and they could become a valuable cooperator.

3.3 Economic benefit and industry significance

The maintenance of comprehensive germplasm collections of fruit and vegetables is a service provided for the direct benefit of researchers, both public and private, and ultimately the respective horticultural industries. Germplasm accessions originating from many countries throughout the world are the source of new genes for disease or pest resistance, drought tolerance, fruit quality, etc. Access to new sources of genetic variability from overseas collections will become more difficult in the future as governments throughout the world recognise plant genetic resources as valuable assets. Therefore, Australia should maintain adequate working germplasm collections of all fruit and vegetable crops.

3.4 Project aims

The aims of this project are to establish a national tomato germplasm collection as a service to all researchers in Australia.

The overall objective in the next 5 to 10 years is to take a national approach towards the conservation of tropical horticultural germplasm. More specifically a database will be established and maintained to record passport and characterisation data on all tropical fruit and vegetable germplasm collections in Australia. Secondly, all tropical fruit and vegetable germplasm accessions which can be stored as seed will be regenerated, characterised, catalogued, and placed in long-term storage at the Centre at Biloela. This project proposal is specifically for the conservation of tomato germplasm.

4 Materials, Methods & Results

4.1 Acquisition of germplasm accessions

The tomato germplasm collection of 1215 accessions stored at QDPI Bowen Research Station and the tomato germplasm collection of 339 accessions held at Redlands Research Station were transferred to the Australian Tropical Crops Genetic Resource Centre at Biloela where the seed was placed in the -20°C seed store. Passport data (species, cultivar name, donor) and evaluation data (disease resistance & susceptibility, agronomic remarks) in a database format was also obtained for the collection from Bowen.

All accessions were catalogued into the Genetic Resource Centre collection by entering passport and inventory information into the database and placing the seed in the -20°C seed store.

4.2 Viability testing.

590 accessions with adequate quantities of seed (>1000 seeds) were tested for seed viability using the rolled-towel method. Seeds were categorised in four ways: normal, fresh, abnormal, or dead. Viable seed includes both normal and fresh seed. Normal seeds grow into normal seedlings; whereas fresh seed is alive and imbibes water but fails to grow into seedlings because of some form of dormancy. Abnormal and dead seed were classified as non-viable. A large number of accessions (42% of total number) had >50% normal seed. The proportion of fresh seed within the accessions ranged from 0 – 100%, and a large number of accessions (42% of total number) had >50% fresh seed. Therefore, fresh seed is an important factor in this tomato germplasm collection.

The remaining 964 accessions with seed quantities of <1000 seeds were not initially tested for viability. Seed quantities ranged from 4 – 990 seeds with 62 accessions having less than 50 seeds.

Therefore, a method of breaking the dormancy of fresh seed was required in order to increase the rate of germination. This was essential before regeneration could be attempted on those accessions where only a very small seed sample remained. For accessions with low

germination rates there was the distinct possibility of losing much of the genetic variability within the accession or even losing the whole accession completely.

4.3 Germinating fresh seed

4.3.1 Materials & methods

The first priority was to develop a procedure for germinating fresh seed that could be used to regenerate accessions with low quantities of seed and accessions with high proportions of fresh seed. A number of experiments were conducted to investigate this matter.

Two seed lots used for the majority of the experiments were Accession 164 (variety Campbell 1327) and Accession 515 (variety PP006). Both seed lots had high levels of fresh seed (Table 1). In addition a commercial variety (Yates Grosse Lisse) was used as a control. 20 seeds of each accession were generally used in each test and these were left to germinate for 14 days whereupon the readings were taken.

Table 1. Characterisation of seeds used in germination tests.

accession #	normal	abnormal	fresh	dead
164	64%	0%	16%	4%
515	32%	2%	56%	10%

The initial tests carried out were those advised in The Association of Official Seed Analysts Journal of Seed Technology (1). These involved the use of 0.2% KNO_3 solution, prepared by dissolving 2g KNO_3 in 1 litre of water, to saturate the germination substrate at the beginning of the test (2), compared with distilled water. Three different substrates were used; paper towel rolls (3) wrapped in towelling material, potting mix made up at Biloela Research Station, and filter paper in petri dishes. Each substrate being moistened before the addition of seeds.

These tests were performed in an incubator using two different temperature and light treatments;

(i) 20°C for 16 hours and 30°C for 8 hours with light for 12 hours (throughout the 30°C regime) and dark for 12 hours.

(ii) 20°C for 10 hours and 30°C for 14 hours with light for 14 hours (throughout the 30°C regime) and dark for 10 hours.

A potting mix was also used to test seeds in the glasshouse.

Additional treatments carried out on seeds placed in the incubator with the settings of (ii) included:

Pre-chilling. The seeds were placed in contact with moist paper towel substrate (distilled water or 0.2% KNO_3) and kept at a low temperature (5°C) for an initial period (7 days) before

they were moved to the incubator (2). These seeds were only used with the paper towel roll test.

Acid Treatment. Seeds were soaked in 10% hydrochloric acid for 2 minutes. They were then rinsed three times in distilled water. These were only used with the filter paper test.

Scarification. Seeds were scarified manually (nicking) which was achieved by cutting away a small portion of the seed coat (4). This followed sterilisation in 1% sodium hypochlorite (made fresh each time it was used) and Brij 35 (3 drops for 10 ml sodium hypochlorite) for 5-20 minutes depending on the size and roughness of the seed. These were only used with filter paper test and 3mM gibberellic acid was used as a comparison to water. Chilling for 3 days at 5°C was also used in conjunction with scarification.

One of the first experiments conducted (Table 2) was to confirm if fresh seeds were germinating or had some form of dormancy. Seeds of the accessions in Table 2 were left to germinate in the incubator with the settings of (ii) either using the paper towel rolls or potting mix.

Table 2. Accessions used to determine viability of fresh seed.

accession #	normal	abnormal	fresh	dead
33	6%	0%	14%	80%
348	0%	0%	0%	100%
434	100%	0%	0%	0%
865	100%	0%	0%	0%
999	0%	0%	100%	0%
1014	0%	0%	100%	0%

The next group of experiments was designed to identify the most suitable culture medium to germinate fresh seeds.

Several different media were used to promote germination; agar on its own, MS salts (5), and MS salts with 1.2 or 2.4 g/l NaCl in order to attempt germination. The pH of each media was adjusted to 5.8 prior to addition of the agar using HCl and NaOH. Agar was added at 8 g/l and dissolved into the media on a hot plate. This media was then sterilised in a pressure cooker for 15-20 minutes and poured into petri dishes in the laminar flow using aseptic techniques.

Seeds were sterilised in 1% sodium hypochlorite (freshly made) prior to culture for 5-20 minutes depending on the size and roughness of the seed. They were then rinsed three times in sterile distilled water.

Those seeds not nicked were placed straight onto the media using aseptic techniques in the laminar flow cabinet. Those that were nicked were cut with a sterilised scalpel, being careful not to damage the endosperm, in the laminar flow cabinet before being placed onto the media. They were then left in the incubator with the settings of (ii).

4.3.2 Results

All results for germination rates were taken 14 days after the seeds were initiated to germinate.

Table 3. Germination rates of accessions to determine viability of fresh seed.

accession #	substrate			
	paper towel rolls		potting mix	
	distilled water	0.2% KNO_3	distilled water	0.2% KNO_3
commercial	90%	65%	95%	80%
33	10%	0%	5%	10%
348	0%	0%	0%	0%
434	75%	35%	90%	80%
865	100%	60%	60%	35%
999	0%	0%	0%	0%
1014	5%	0%	0%	0%

Table 4. Germination rates using paper towel rolls as a substrate.

	incubator	commercial	164	515
distilled water	(i)	100%	55%	30%
	(ii)	95%	35%	15%
0.2% KNO_3	(i)	100%	35%	30%
	(ii)	100%	55%	35%

Table 5. Germination rates using potting mix as a substrate.

	incubator	commercial	164	515
distilled water	(i)	94%	44%	11%
	(ii)	95%	80%	35%
0.2% KNO_3	(i)	100%	65%	35%
	(ii)	100%	65%	20%

Table 6. Germination rates using potting mix as a substrate in the glasshouse.

	commercial	164	515
distilled water	100%	50%	10%
0.2% KNO_3	100%	60%	35%

Table 7. Germination rates using roll paper towel substrate, following prechill; incubator (ii)

	Commercial	164	515
distilled water	100%	45%	15%
0.2%KNO_3	90%	40%	10%

Table 8. Germination rates using filter paper as substrate; incubator (ii).

treatment		commercial	164	515
acid scarified	distilled water	100%	35%	25%
	0.2% KNO_3	100%	50%	15%
sterilised	distilled water	100%	65%	10%
	3mM GA_3	100%	50%	50%
sterilise & nick	distilled water	90%	100%	90%
	3mM GA_3	100%	95%	100%
sterilise & chill	distilled water	95%	55%	35%
	3mM GA_3	100%	40%	45%
ster./nick/chill	distilled water	100%	100%	95%
	3mM GA_3	90%	95%	100%

Table 9. Germination rates using culture media as substrate.

treatment	media	commercial	164	348	515
scarified	agar only	100%	100%	0%	100%
	MS salts	100%	100%	0%	95%
	MS + NaCl[(1)]	75%	30%		25%
	MS + NaCl[(2)]	65%	5%	0%	0%
non-scarified	agar only	100%	50%	0%	20%
	MS salts	100%	55%	0%	25%
	MS + NaCl[(1)]	95%	0%		0%
	MS + NaCl[(2)]	60%	0%	0%	0%

(1) 1.2 g/l NaCl, (2) 2.4 g/l NaCl

4.4 Regeneration of accessions

The program to regenerate tomato germplasm accessions started with the *Lycopersicon esculentum* accessions with small numbers of seed. All seeds were germinated and grown using culture method identified in the early experiments. After obtaining confidence with *L. esculentum* accessions, the seed culture method was tried with wild *Lycopersicon* species.

The seed culture method increased germination considerably, with all accessions tested having greater than 40% germination by day 5, and greater than 85% germination after 2 weeks in the culture tubes. Tubes were incubated at 20/30°C for 16/8 hours with daylenths of 12 hours until the seedlings reached the 4-leaf stage.

Seedlings were then transferred to the greenhouse for one week in their tubes to harden-off before being transplanted out at the 4-6 leaf stage into pots containing a rich potting mix. The major problem encountered in the greenhouse was powdery mildew infection, so all plants were routinely sprayed with fungicide every fortnight to control powder mildew.

Some of the wild *Lycopersicon* species did not self pollinate naturally, and a mechanical vibrator was used to increased the rate of pollination. However, seed set was low and seed collection continued over several months in order to collect sufficient quantities of seed.

Seed was extracted from the fruit by adding 1.5% HCl to the pulp and incubated for 1.5 hours at room temperature. The seed was then washed through a sieve and the seed dried over night. A higher concentration of HCl or a longer fermentation time successfully removed the pulp from the seed but significantly reduced the viability of the seed.

4.5 Storage and maintenance of germplasm collection

After extracting seed from the fruit the seed sample was dried slowly (over a period of one month) in a drying room operating at 15°C and 15% relative humidity until the seed reached 6% moisture content.

The seed sample was then placed in laminated alfoil packet and entered into the -20°C long-term seed store where the seed sample should remain viable for 50-100 years.

A viability test was conducted on every sample entering the seed store using the procedures of the Association of Official Seed Analysts (1, 2). All samples in the seed will be monitored for seed viability every 10 years or sooner if required.

5 Discussion

5.1 Germinating fresh seed

The original aim of the project was to regenerate those accessions with low quantities of seed or reduced viability rates. Normally this is a routine procedure. However, after conducting viability tests on the germplasm accessions it was found that approximately half the accessions had significant proportions of "fresh" seed which is a form of dormancy preventing germination. Therefore, the major aim of the project was re-focused to develop a procedure to germinate "fresh" seed of tomato. This was essential before regeneration could be attempted on those accessions with only very small seed samples. For accessions with low germination rates there was the distinct possibility of losing much of the genetic variability within the accession or even losing the whole accession completely.

Fresh seed of tomato does not germinate in the absence of any treatment to promote germination (Table 3). Accession numbers 999 and 1014, which had 100% fresh seed, had no germination; except for one test with accession 1014 where 5% germination was obtained with paper towel rolls soaked in distilled water. Therefore, the problem is how to promote germination of "fresh" seeds that have some form of dormancy.

The problem was solved using the technique of seed nicking. This was achieved by cutting the seed coat with a scalpel following sterilisation. This was the only technique that achieved any significant increase in germination (Table 8). There was no significant difference caused by any other treatment including chilling, acid scarification & sterilisation (Tables 7 & 8). In addition, the different solutions (distilled water, gibberellic acid and potassium nitrate) used to wet the substrates appeared to make no difference to germination rates (Tables 3, 4, 5, 6, 7 & 8).

Germinating seeds on culture media produced visually healthier seedlings than those obtained on filter paper. Advantageously, no constant watering was required and the seeds were left from the time they were placed on the media to the time they reach a size for transplanting. When culture media was used to germinate seeds, which is an ideal media to germinate seeds, it was still found that scarifying the seeds by nicking significant increased germination rates. The culture media selected to germinate and grow the tomato seedlings was similar to that

recommended by El-Farash et al (6), but with sugar omitted to reduce problems from fungal contamination. The media was: MS salts supplemented with (mg/l) 6 IAA, 5 kinetin, 40 adenine sulfate, 170 $NaH_2PO_4.H_2O$, 100 i-inositol, 0.1 thiamine HCl, 0.5 pyridoxine, 0.5 nicotinic acid, and agar (0.6 g/l). The addition of sodium chloride to the MS salts appeared to inhibit germination rather than increase it (Table 9).

5.2 Regeneration of accessions

The current status of the national tomato germplasm collection is listed in Table 10. 70% of the accessions have sufficient quantities of viable seeds in storage. The other 30% of the accessions will require regeneration within the next few years. The first priority should be on regeneration of the wild species.

Table 10. Current status of tomato germplasm collection.

species	total number of accessions	number of accessions with viable seed quantities	number of accessions requiring regeneration
L. cheesmaniae	3	3	0
L. chmielewskii	1	0	1
L. esculentum var *esculentum*	926	655	271
L. glandulosum	20	12	8
L. hirsutum	11	8	3
L. parviflorum	1	1	0
L. pennellii	7	2	5
L peruvianum	32	27	5
L. pimpinellifolium	106	86	20
L. species	9	6	3
L. esculentum.hirsutum	1	1	0
L. esculentum.pennellii	1	0	1
L. esculentum.peruvianum	47	10	37
L. esculentum.pimpinellifolium	44	31	13
L. hirsutum.pimpinellifolium	7	6	1
totals	1216	848	368

5.3 Maintenance of tomato germplasm collection

The Australian Tropical Crops Genetic Resource Centre at Biloela will continue to maintain the national tomato germplasm collection. All accessions will be maintained in long-term storage at the –20°C. Viability of seed samples will be monitored regularly every 10 years and accessions will be regenerated as required.

Small samples of seed (50 seeds) of germplasm accessions are freely available to researchers on request.

Passport, characterisation & evaluation, and inventory data on all accessions will be maintained on the database operated by the Genetic Resource Centre. The passport and characterisation & evaluation information can be accessed by clients via the internet (http://www.dpi.qld.gov.au/auspgris/). AusPGRIS is the Australian Plant Genetic Resources Information System established by QDPI under a GRDC funded project. It is a national system for all *ex-situ* plant genetic resources maintained in Australia.

5.4 Viability testing of "fresh" seed

Plans are under-way to conduct viability tests on new seed samples to identify the percentage of "fresh" seed and compare this with that of the parent population. These tests will be conducted over different time intervals. From these results it may be possible to detect whether: (i) there is a genetic effect causing production of "fresh" seed; or (ii) if the high proportion of "fresh" seed was due to the conditions the seed was stored previously; or (iii) if long-term storage at -20°C affects seed dormancy. If the new seed shows a high percentage of normal seed compared to "fresh" seed there may not be any requirement to scarify the seeds the next time accessions require regeneration.

6 Recommendations

- The national tomato germplasm collection continued to be maintained by the Australian Tropical Crops Genetic Resource Centre at Biloela.

- The Australian Tropical Crops Genetic Resource Centre conduct further research to determine if "fresh" seed is a genetic problem related to certain tomato accessions, is a problem related to long-term storage, or is not a problem under the present the -20°C storage conditions.

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