Australian Government
Rural Industries Research and Development Corporation

Condiment Paprika Breeding

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January 2008

RIRDC Publication No 07/126
RIRDC Project No US-136A

ISBN 1 74151 523 8
ISSN 1440-6845

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Published to Web in January 2008

Foreword

An increased demand for natural spices and colorants has expanded the demand for high quality condiment paprika worldwide. Hungary has produced high quality condiment paprika in the past, but the exports from that country have declined significantly over the last 15 years. By using Hungarian cultivars and other genetic material in a breeding program for Australia it is hoped to establish a viable condiment paprika industry in Australia.

To achieve these aims a program has been established by RIRDC which is a 3-year continuation of a previous project (US-116A: *Condiment paprika breeding and hybrid seed production*). The aims are to produce cultivars with high initial pigment and dry matter content suitable for direct seeding and mechanical harvesting. A further aim is the development of a hybrid system so that hybrid seed can be produced at low cost, giving paprika growers the benefit of the resultant heterosis (hybrid vigour).

This report concentrates on the results of the paprika plant-breeding. One new cultivar has been reported here and several promising lines identified for potential release.

This project was funded from RIRDC core funds which are provided by the Australian Government.

This report is an addition to RIRDC's diverse range of over 1700 research publications. It forms part of our New Plant Products R&D Program which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

Thanks are due to Adjunct Professor Nick Derera AM of ASAS Pty Ltd who has been a key driver for the establishment of a viable paprika industry in Australia.

This project was built upon the findings of the previous paprika project, and the contributions of Sue Fiffer and Fran Ebb, both Technical Officers, are recognized. The significant contribution and commitment of Adriana Hoxha, the Technical Officer responsible for the current project, is greatly appreciated. Thanks are also due to Dr Natalia Nagy for permitting the use of her seed lines in our further research. In addition, the cooperation and support from James Bell and Graham Brown at the Plant Breeding Institute is gratefully acknowledged.

We would also like to thank Dr. John Vella of Speedy Seedlings Pty. Ltd for cooperation and support of commercial trials.

Professor P. W. Bosland, Dr C. Shifriss, Dr N. Somogyi and Dr. Jae-Hyung Yoo are thanked for providing plant material.

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Executive Summary

What this report is about
This report is a three-year continuation of a previous RIRDC project, US-116A: *Condiment paprika breeding and hybrid seed production*). Condiment paprika, which is the dried and ground flesh of speciality types of Capsicum species, is a globally traded commodity. During the project, breeding lines were developed using single seed descent (SSD), selections made and new germplasm with specific traits introduced into the breeding program. A promising future for capsicum hybrid seed production in Australia is apparent.

Target readership
The report intends to inform current and potential paprika growers and breeders of paprika of our progress towards the goal of providing the industry with superior material. In addition, we wish to inform these same groups of our progress towards developing efficient hybrid seed systems for capsicum.

Objectives
The objectives of the project were as follows:

1. Development of commercial cultivars of paprika for Australia, with a high proportion of the total yield in the first harvest, high dry matter content and high pigment content.
2. Provision of an alternative crop to farmers, especially vegetable growers.
3. Development of a paprika industry chain that will provide a new industry for Australia.
4. The development of breeding methods for rapid generation advancement.
5. Investigation and use of male sterility as a method of hybrid seed production.
6. Investigation of vegetative propagation of male sterile lines as a cost effective way to maintain male sterile stocks.

Methods used
Standard plant breeding technology has been employed such as crossing, selection, yield and pigment testing. First visual selection was employed through observations of the plant type in the field, with selection for upright plants with pendulous (hanging and swinging freely) paprika-type fruits, where the majority of fruit ripened at the same time; the fruit is detachable from the calyx and easily snaps-off from the stem. These characteristics are important for machine-harvest ability. Advanced lines carrying the detachability gene, identified in the previous project, have been field tested and further selected. High detachability refers to the easy removal of the fruit from the green fleshy calyx, while the snap-off trait relates to a fruit “stem” that easily breaks. Field trials of relatively stable lines were conducted to assess yield and to provide samples for quality assessment. Further testing and selection was conducted before seed increase and entry into commercial trials in grower’s fields. The key selection criteria were fruit yield, high quality (colour, aroma and taste), disease resistance and suitability of the crop for machine harvest.

The single seed descent (SSD) method was employed as a rapid technique for advancing generations for the first two years of the project. The SSD method involves growing single seeds from each population in each generation in small containers in the glasshouse, so that only a few fruit are formed quickly, and 2-3 generations can be grown per year. Following prior agreement SSD was discontinued in the final year of the project. This freed resources for an exploration of hybrid seed production based on male sterility (ms).

An important non-visual selection characteristic of paprika is the pigment concentration in the fruit. This is measured using American Spice Trade Association units (ASTA). All the ASTA tests were conducted at Plant Breeder’s Institute-Cobbitty (PBI-Cobbitty), using the methodology developed in the previous project.

The planned examination of capsicum hybrid seed production is an extension of work begun by an earlier PhD student, who developed genetically ms materials. To increase the frequency of ms plants in a given population, intermediate crosses were made between ms individuals in the F2 generation and F1 plants carrying the recessive gene for ms. Segregation of F2 ms was observed and the presence of the gene ms3 was confirmed by pollen staining and pollen germination. Vegetative propagation was used to maintain the ms lines produced. Pollen staining, pollen germination, *in vitro* vegetative propagation and protein identification of ms lines was conducted in the laboratory.

Results

The continuation of conventional breeding led to the release of the cultivar "Cerise Sweet" with good paprika characteristics (high dry matter % and high extractable pigment content). In addition, several promising lines selected from stage 3 of the variety and strain trial (VS3) have been identified for possible Plant Breeders Rights (PBR) protection.

The SSD method worked well, enabling interspecific cross material to progress quickly to field trial. Promising materials with paprika-like quality and excellent detachability were identified.

To establish a working system for the production of F1 hybrid capsicum seed two main issues must be addressed; these are increased frequency of ms plants in a given population and cost effective maintenance of ms stocks. In the first instance, male sterile plants were crossed with fertile plants derived from the same parent combination. In the second instance, ms individuals were propagated by cuttings. The cost effectiveness of this method of propagation is calculated and discussed later.

The findings of this study have application beyond paprika; they apply to the whole Capsicum genus, including the vegetable and ornamental sectors.

The Implications for stakeholders

Improved paprika cultivars, paprika germplasm, better breeding and propagation methods arising from this project are available to all stakeholders. The new interspecific materials developed can be advanced and used to breed paprika cultivars, alternatively they may be commercialised following further evaluation. The hybrid seed production results and the vegetative propagation of ms lines provide a basis for the establishment of a commercial hybrid seed production industry in Australia. This would not be restricted to paprika alone, but could be applied across all Capsicum species.

Recommendations

The new cultivar "Cerise Sweet" protected by PBR, offers the industry significant advantages and could be promoted to interested growers. A commercial partner will need to be found to undertake the bulking up and commercialisation of "Cerise Sweet" and the submission of other promising lines for PBR testing.

Unfortunately, the paprika market is small in Australia and unlikely to grow significantly. In addition, the infrastructure required to produce dried and crushed condiment paprika for retail sale is expensive and not currently available to most growers. The hybrid F1 seed results are promising and a cost effective F1 hybrid seed production system can be established and applied to the much larger vegetable capsicum industry. However, combining ability and the identification of optimal heterotic combinations are still to be established.

Vegetable capsicum production is an important industry in Australia and hybrid seed is imported at significant cost from overseas suppliers. The findings of this research indicate that hybrid capsicum seed production is likely to be successful in Australia. We recommend that further work be undertaken to examine the transferability of these findings to vegetable capsicum production in Australia.

Introduction

This project is a 3-year extension of the RIRDC project US-116A: *Condiment paprika: breeding, processing and commercialisation (Stage 2)*, which continued breeding paprika cultivars for Australian production. The previous project identified sources of material for possible cultivar development and these were used as the basis of the current project. Earlier findings identified machine harvestability as a key constraint to the expansion of paprika production in Australia. This is because Australian labour costs are high and growers are unable to compete with low-cost labour in Africa and Asia.

The breeding and research program was therefore focused on developing machine harvestable germplasm and cultivars.

To develop a competitive paprika industry, higher productivity is required. This can be achieved by exploiting hybrid vigour. The feasibility of hybrid seed production was investigated using genetic male sterile (ms) paprika lines and vegetative propagation of these ms stocks. This work builds on the earlier findings of Dr. N. Nagy who worked in the precursor project.

An effective hybrid seed production scheme was developed and promising results have been achieved, particularly on the propagation of male sterile lines. However, further development of this seed production scheme is required.

The Breeding Program

Crossing nursery

A standard crossing nursery was developed to introduce traits considered important for the machine harvesting of paprika cultivars in the field. The traits relate to detachability of the fruit in the field when ripe. Most wild Capsicum species have the "detachability" trait or the easy removal of the fruit from the calyx. The fruit snaps off by breaking the pedicel between the calyx and the stem. Initially, the crossing nursery focussed on combining disease resistance with the detachability trait. The primary diseases targeted in crossing were Tobacco Mosaic Virus and *Phytophthora spp.*

Capsicum chacoense was used as a source of the detachability trait. Interspecific crosses were made with Hungarian and American paprika cultivars (largely from New Mexico). In most cases the F1 plants produced were male sterile. However, the fruits of these hybrids were too soft for machine harvesting. *C. chacoense* and various wild species were used as new sources of detachability in crosses. These species have the detachability and snap off pedicel genes. They were crossed with *C. baccatum* var *pendulum* to create an intermediate form, which could then be crossed with paprika cultivars.

In South America, *C. baccatum* is the most commonly grown species, where it is called 'Aji'. Three botanical varieties are recognized: *C. baccatum var. baccatum, C. baccatum* var. *pendulum,* and *C. baccatum var. microcarpum. C. baccatum* flowers have yellow, brown, or dark green spots on the corolla. Fruits vary in pungency from non-pungent to very hot. They embody unique aromatics and flavours that can be overpowering to some people. This chilli is used to make marinated fish. Aji Amarillo is the most common *C. baccatum* in Peru. In the United States it is sometimes called 'Yellow Pepper". The pods are 10-15 cm long and deep orange colour when ripe. This pod type has been known in Peru since ancient Inca times, where it is represented in drawings and pottery.

Interspecific material produced

During 2004-2005 the crossing nursery was placed in the double tunnel house to avoid cross-pollination and bird damage that occurs in open areas. Both interspecific crosses and crosses for disease resistance were made during this season. Interspecific combinations involved *C. chacoense, C. baccatum, C. aviculare, C. frutescents* and other *C. annum* cultivars, which were then "top-crossed" by other accessions and/or among each other. Embryo abortion caused by post-fertilisation interspecific incompatibility was a genetic barrier in these crosses. To overcome this barrier, embryo rescue and bridging crosses were employed. Hybrid sterility was an additional genetic barrier. This sterility prevented the production of the next generation as some crosses failed to set seed. Table 1 (below) lists the material produced and the crosses where the embryo abortion took place.

Fig.1: **Crossing nursery 2004-2005**

Table 1: **List of successful crosses and aborted embryos 2004-2005**

Nr	Pedigree	No. of seeds
1	C.bacc.pendulum/Mihalyteleki	2
2	C.bacc.pendulum/C.frutescents 414	4
3	C.bacc.pend/Kalocsai 801//C.frutescents 414	2
4	C.bacc.pend/Kalocsai 801//C.frutescents 414	2
5	C.bacc.pend/Kalocsai 801//C.frutescents 414	3
6	C.bacc.pend/Kalocsai 801//C.frutescents 414	3
7	C.bacc.pend/Kalocsai 801//C.frutescents 414	3
8	C.aviculare/C.frutescents 414	8
9	C.aviculare/C.frutescents 414	5
10	C.chacoense/C.frutescents 414	5
11	C.chacoense/C.frutescents 414	3
12	C.chacoense/C.frutescents.414	13
13	C.chacoense/Co.57-13.4b	4
14	Co57-13.3b/C.bacc.pendulum	3
15	Co57-13.3b/C.bacc.pendulum	5
16	Sud Africa/Co57-13.3b	10
17	Sud Africa/Co57-13.3b	15
18	Sud Africa/Parpi queen	30
19	Sud Africa/Papri queen	20
20	Sud Africa/Cerise sweet	3
21	Sud Africa/Cerise sweet	25
22	Sud Africa/Cerise sweet	1
23	Sunired//Aji Amarilo C.bacc./C.frutescents 414///Sunired	5
24	Sunired/C.bacc.pendulum	8
25	Co.57-13.4b/C.bacc.pend.//C.frutescents 414	1
26	Co.57-13.4b/C.bacc.pend.//C.frutescents.414	1
27	Co.57-13.4b/C.bacc.pend.//C.frutescents 414	2
28	Co.57-13.4b/C.bacc.pend.//C.frutescents 414	2
29	Co57-13.4b/C.bacc.pend.///Aji Amarilo C.bacc/C.frutes 414.//Aji Amarilo C.bacc./P.Queen	2
30	Co57-13.4b/C.bacc.pend.///Aji Amarilo C.bacc/C.frutesc 414.//Aji Amarilo C.bacc./P.Queen	2
31	Co57-13.4b/C.bacc.pend.///Aji Amarilo C.bacc/C.frutesc414//Aji Amarilo C.bacc./P.Queen	2
32	PM 1231 (R:Phyto) Chourbadjiidski/Earlysuni	12
33	Earlysuni/Carolina Caynne Res. M incognita rac.1,2,3 and 4	8
34	Sunired/Carolina Caynne Res. M incognita rac.1,2,3 and 4	23
35	Carolina Caynne Res. M incognita rac.1,2,3 and 4/Papri King	10
36	Joe E. Parker Res TMV (0)/Sunired	10
37	Joe E. Parker Res TMV (0)/Sunired	12
38	Joe E. Parker Res TMV (0)/Sunired	13
39	Co57-13.3b/Carolina Caynne Res. M incognita rac.1,2,3 and 4	10
40	C.bacc.pendulum/Mihalyteleki	Aborted embryos
41	C.bacc.pendulum/Mihalyteleki	"
42	C.bacc.pendulum/Co.57-13.4b	"
43	C.bacc.pendulum/C.Cardenasi	"
44	C.bacc.pendulum/C.Cardenasi	"
45	C.bacc.pendulum/Co.57-13.4b	"
46	C.bacc.pendulum/Co.57-13.4b	"
47	C.bacc.pendulum/C.frutescents 414101	"
48	Papri king/C.bacc.pendulum	"
49	Co57-13.3b/C.bacc.pendulum	"
50	Co57-13.3b/C.bacc.pendulum	"
51	Co57-13.3b/C.bacc.pendulum	"

During 2005-2006, the crossing program again targeted disease resistance and interspecific combinations. New accessions of wild species and disease resistance sources were received from the Centre for Genetic Resources (CGR) in the Netherlands (Table 2). These new disease resistant sources were crossed with paprika cultivars to initiate the development of resistant paprika cultivars. The focus on disease arose from the high incidence of disease infection during the previous two seasons. A decision was taken to search for disease resistance in the world Capsicum collections. The following varieties (Table 2), with various levels of disease resistance, were introduced into our collection. Table 3 contains other disease resistant lines used as sources of resistance in crosses.

Table 2: **Wild species and disease resistant lines received from the Centre for Genetic Resources (CGR) , the Netherlands**

Acc. #	CGN #	Species	Accession name	Country of origin	Year
322	22770	C. annuum	401	Nicaragua	11/02
323	22773	C. annuum	7101	Guatemala	11/02
324	16974	C. annuum	AC 1534	Mexico	08/01
325	16976	C. annuum	AC 2255	Honduras	08/01
326	21467	C. baccatum var. baccatum	AC 1230	Brazil	10/00
327	17025	C. baccatum var. baccatum	No. 1553; PI 281306	Bolivia	10/95
328	23278	C. baccatum var. baccatum	PI 337524	Argentina	10/03
329	23205	C. baccatum	SA 326	Bolivia	10/03
330	20510	C. chacoense	342	Paraguay	06/01
331	22790	C. frutescens	AC 1249	Mexico	11/02
332	23210	C. frutescens	SA 137; PI 257155	Peru	10/03
333	22208	C. galapagoense	AC 1501		10/04
334	22794	C. praetermissum	SA 221; PI 260595, Goat pepper	Brazil	10/02
335	19198	C. praetermissum			08/01
336	22876	C. tovarii	PI 606708, NMCA 90008	Peru	10/02
337	17181	C. annuum	Early Calwonder-selection	Res. Tomato TMV	10/95
338	20806	C. chinense	RU 72-292	Res. L1	09/99
339	21546	C. frutescens	Tabasco	Res. L2	10/00
340	17004	C. chinense	Landrace/Traditional cultivar	Res. L3	07/01
341	17036	C. chinense	Lemon drop selection	Res. L3	09/97
342	20801	C. chinense	PI 257117 selection	Res. L3	09/99
343	21557	C. chinense	Res. TSWV	Res. L3	10/00
344	22788	C. chinense	Landrace/Traditional cultivar	Res. L3	10/02
345	22829	C. chinense	Miscucho colorado, Res. TSWV	Res. L3	12/03
346	21477	C. chacoense	Res. P8	Res. L4	10/00
347	22869	C. chacoense	Res. P8	Res. L4	11/02

Table 3: **Other disease resistance sources used in crosses**

Acc. Nr	Name
115	NM Twilight Res. CMV sgr
116	Joe E. Parker TMV (0) resist.
117	Carolina Cayenne Res. M. incognita rac.1,2,3 & 4
118	Crillio De Morals NMCA10467 (CM-334) Phytophthora resist.
119	PI.215699 (Selection) Verticillium wilt resist.
220	Crillio De Moral's. Phytophthora resist.
136	PM 1231 Chourbadjiiski. Phytophthora resist.
172	Lilac (F1) TMV resist.

Successful crosses produced using these disease resistant parents were sown in the field trial the next season (table 4).

Table 4: **Successful crosses 2005-2006**

CO5	Pedigree	# of seed
12	C.chacoense/C.frutescents.414	13
16	Sud Africa/Co57-13.3b	10
17	Sud Africa/Co57-13.3b	15
18	Sud Africa/Papri Queen	30
19	Sud Africa/Papri Queen	20
20,21	Sud Africa/Cerise Sweet	29
32	PM 1231 (R:Phyto) Chourbadjiidski/Earlysuni	12
D28	Earlysuni/Carolina Caynne Res. M incognita rac.1,2,3, 4	8
D33	Sunired/Carolina Caynne Res. M incognita rac.1,2,3 and 4	23
D6	Carolina Caynne Res. M incognita rac.1,2,3 and 4/P. King	10
D16	Joe E. Parker Res TMV (0)/Sunired	10
D15	Joe E. Parker Res TMV (0)/Sunired	12
D14	Joe E. Parker Res TMV (0)/Sunired	13
D43	Co57-13.3b/Carolina Caynne Res. M incognita rac. 1,2,3,4	10
CO4.66.1	Sunired//Aji amarilo C.bacc./C.eximium	10
CO4.68.1	C.Chinense.Jacq./P.mild	7
CO3.15.1.1	Mihalyteleti/C.bacc.pend.	13

Selection of cultivars

Initial genotypes selected for cultivar release came from the material developed in the previous project. Variety and strain trials were undertaken each year during the growing season to identify candidates for release. Key selection traits included yield, dry matter content, ASTA, uniformity, growth habit, high fruit set, pendulous fruit and early ripening.

Variety and strain trial 2004-2005

Fifty one lines were included in the first variety & strain (VS1) trials in 2004-2005 and the most promising candidates selected. Field evaluation was conducted using a 1-10 score for plant growth habit, uniformity and stability. Those lines that did not display uniformity were eliminated. Of the 51 lines included in this trial 42 were selected for further testing in the laboratory (Table 5).

Table 5: **Field evaluation of first variety & strain (VS1) trial 2004-2005**

Code/04	Pedigree	Pedigree code	Score
L1	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op1(c).1	5.8
L2	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op1(c).2	7.8
L3	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op2(b).1	6.5
L4	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op2(b).2	6.8
L5	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op2(a).1	5.8
L6	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op2(b).1	5.5
L7	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op2(b).2	7.5
L8	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op3(a).1	6.8
L9	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op3(a).2	6.3
L10	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op4(c).1	8.3
L11	C97.15/ Szegedi 20	C98.65.1.1.1.1op1(a).2	4.5
L12	C97.15/ Szegedi 20	C98.65.1.1.1.1op1(b).1	6.3
L13	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).1	4.3
L14	C97.15/ Szegedi 20	C98.65.1.1.1.2op2(b).1	3.5
L15	C97.15/ Szegedi 20	C98.65.1.1.1.2op2(c).2	4.0
L16	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).1	6.5
L17	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).2	7.5
L18	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).3	3.8
L19	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).4	6.0
L20	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(a).1	4.0
L21	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(b).1	6.0
Ca30	Sz57-13		7.0
L23	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(c).1	6.0
L24	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(c).2	4.3
L25	C97.15.op.22/Sz80	C99.209.2(c).1.1op1(a).1	4.3
Ca233 ('03)	Co.57-13.4.b (2003)		7.0
Ca233 ('04)	Co.57-13.4.b (2004)		6.8
L28	HM5/Papri Mild	C99.177.2.1.2op1(a).2	Out
L29	Kalochai 801*2/Jalapeno	C98.1.1.1.2.1.2.2	5.5
L30	"	C98.1.1.1.2.2.2.1	7.8
L31	"	C98.1.1.2.1.1.1.1	Out
L32	"	C98.1.1.2.1.1.1.2	Out
L33	"	C98.1.1.9.1.1.1.1	Out
L34	"	C98.1.1.10.2.1.2.1	Out
L35	"	C98.1.1.10.2.1.2.2	Out
L36	Szeggedi 20*2/C.chac	C98.65.1.1.1.1.1.1	4.8
L37	"	C98.65.1.1.1.1.2.1	6.0
L38	"	C98.65.1.1.1.1.2.2	5.5
L39	"	C98.65.1.1.1.2.1.1	6.0
L40	"	C98.65.1.1.1.2.1.2	6.0
L41	"	C98.65.1.1.1.2.2.1	5.3
L42	"	C98.65.1.1.1.2.2.2	3.3
L43	"	C98.65.1.1.3.1.1.1	6.0
L44	"	C98.65.1.1.3.1.1.2	out
L45	"	C98.65.1.2.3.1.1.1	out
L46	"	C98.65.1.2.3.1.2.1	5.0
Ca235 ('03)	Co.57-13.3.b (2003)		6.8
Ca235 ('04)	Co.57-13.3.b (2004)		6.8
L49	HM5/Papri Mild	C99.177.2.1.4.1	out
L51	HM5/Conquistador	C99.180.1.5.1.1	out

Lines that did not display uniformity and lodging resistance were eliminated (marked “out” in the score column). Yield and dry matter content were then measured and the results are shown in Table 6.

Table 6: **Variety and strain (VS1)trial yield and dry matter content 2004-2005**

Line nr.	Pedigree	Pedigree code 04-05	Yield/plant (kg)	% of ripe fruits	Dry matt (%)	ASTA
L1	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op1(c).1	0.371	66	18	196
L2	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op1(c).2	0.352	68	19	192
L3	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op2(b).1	0.317	83	18	233
L4	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op2(b).2	0.297	88	18	199
L5	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op2(a).1	0.330	69	18	179
L6	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op2(b).1	0.395	80	17	185
L7	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op2(b).2	0.390	80	16	177
L8	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op3(a).1	0.265	84	19	218
L9	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op3(a).2	0.247	88	19	206
L10	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.2op4(c).1	0.302	64	16	196
L11	C97.15/ Szegedi 20	C98.65.1.1.1.1op1(a).2	0.295	77	15.7	228
L12	C97.15/ Szegedi 20	C98.65.1.1.1.1op1(b).1	0.330	77	16	242
L13	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).1	0.312	83	15	188
L14	C97.15/ Szegedi 20	C98.65.1.1.1.2op2(b).1	0.440	72	14	187
L15	C97.15/ Szegedi 20	C98.65.1.1.1.2op2(c).2	0.300	60	16	214
L16	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).1	0.272	81	15	218
L17	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).2	0.317	80	16	200
L18	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).3	0.425	76	16	205
L19	C97.15/ Szegedi 20	C98.65.1.1.1.2op1(a).4	0.347	76	15	207
L20	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(a).1	0.322	87	16	224
L21	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(b).1	0.345	81	15	235
L23	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(c).1	0.297	78	16	234
L24	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(c).2	0.305	77	16	218
L25	C97.15.op.22/Sz80	C99.209.2(c).1.1op1(a).1	0.205	82	19	210
L29	Kalochai 801*2/Jalapeno	C98.1.1.1.2.1.2.2	0.42	67	16	219
L30	"	C98.1.1.1.2.2.2.1	0.352	86	18	197
L36	Szeggedi 20*2/C.chac	C98.65.1.1.1.1.1.1	0.255	83	16	260
L37	"	C98.65.1.1.1.1.2.1	0.525	83	17	255
L38	"	C98.65.1.1.1.1.2.2	0.315	88	16	249
L39	"	C98.65.1.1.1.2.1.1	0.465	79	15	199
L40	"	C98.65.1.1.1.2.1.2	0.335	76	15	205
L41	"	C98.65.1.1.1.2.2.1	0.330	75	16	204
L42	"	C98.65.1.1.1.2.2.2	0.297	78	17	214
L43	"	C98.65.1.1.3.1.1.1	0.290	84	17	230
	Mean		0.326	78.25	16.53	212.8
	LSD		0.148	19.36	2.18	48.90

Further selections were made after statistical analyses and the best performing lines were included in the second variety and strain (VS2) trials in the next season.

Variety trial 2005-2006

The 2005/06 season experienced unusually dry conditions and a heavy *Phytophora* infection. As a result, single plant production values rather than plot values were calculated. The purpose of the paprika variety trial was to find suitable parental material for combining ability tests to establish the F1 hybrid seed production system. It was anticipated that the F1 hybrid seed will produce high yielding plants. However, it is important that the parents have the appropriate suite of traits, such as mechanical harvestability, high dry matter and high pigment content. In addition, high *Phytophthora* and virus

infections make it necessary to combine parents with adequate levels of resistance to both diseases. Unfortunately, effective resistance is very rare among the *Capsicum annuum var. annuum* varieties. Therefore resistance must be found among the other *Capsicum* species such as *C. chinense, C. baccatum* etc. This requires difficult to make interspecific crosses and in most cases, bridging crosses. VS1 and VS2 trial results are presented in Tables 7 and 8, respectively.

Table 7: **Cultivars included in VS1 trials, 2005-2006**

Acc.nr	Cultivar	Origin
39	Szegedi 80	Hungary
238	Cerise Sweet	The University of Sydney
229	Sunired	The University of Sydney
40	SzNFD	Hungary
65	Papri Queen	United States of America
37	Kalocsai 801	Hungary
28	Szegedi 20	Hungary
30	Szegedi 57-13	Hungary
64	Papri King	United States of America
32	Szegedi 178	Hungary
173	Pimento	
197	Festival	Hungary
199	Mihalyteleki	Hungary
201	Napfeny	Hungary
222	Jariza	Spain

The following bar charts with their Standard Deviation show the performance for important traits of various cultivars (Figures 2, 3, 4 and 5).

Fig 2. **Percentage of ripe fruit at harvest time for VS1 trial 2004-2005** (see table 7)

Figure 2 (above)indicates the percentage of synchronized ripening of the various cultivars tested. The fruits of the Spanish cultivar Jariza (222) ripen simultaneously, as well, the cultivars Szegedi 80 (39) and Kalocsai 801 (37) are not significantly different from Jariza. All three cultivars lend themselves to mechanical harvesting.

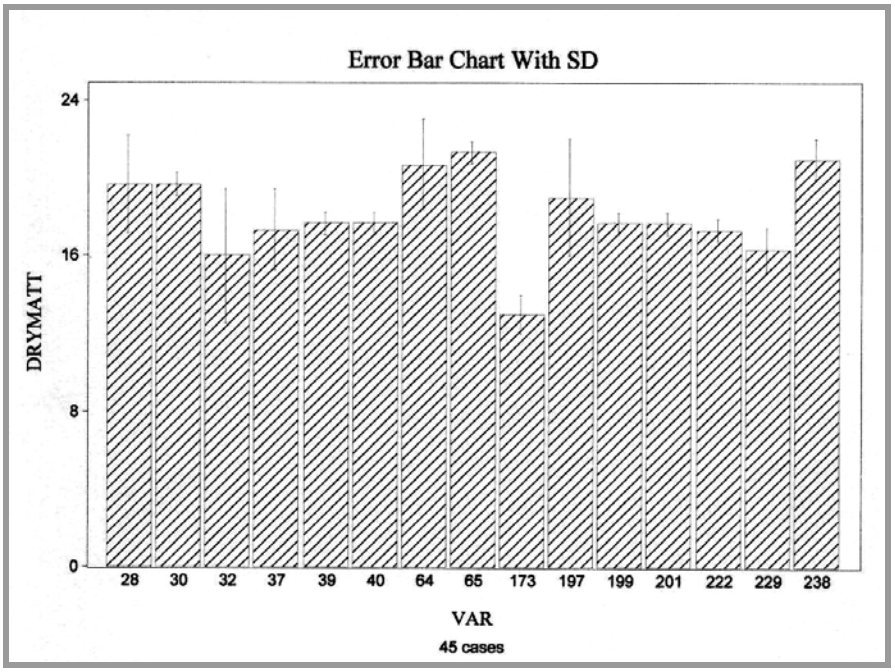


Fig. 3: **Dry matter content for VS1 trial** (see table 7)

Figure 3 (above) presents the variation of dry matter content of the fruits among the tested cultivars. There is a significant difference in dry matter for Pimento (173) only; this cultivar is clearly inferior to all other tested cultivars. The cultivars Szegedi 20 (28), Szegedi 57-13 (300), Papri King (64), Papri Queen (65), Festival (197) and Cerise Sweet (238) are all potential parents.

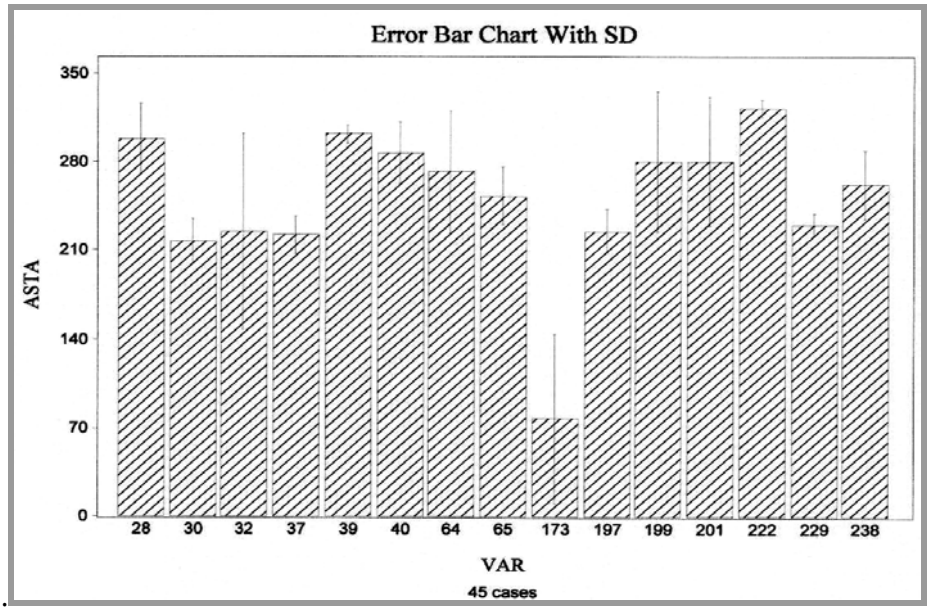


Fig. 4: **Extractable colour content (ASTA) for VS1 trial (see table 7)**

Figure 4 (above) demonstrates the pigment content of the fruits using the international unit of ASTA. This is the most important trait in the spice trade and in oleoresin production. In general all cultivars except Pimento (173) have reasonable pigment production. The better ones are Szegedi 20 (28), Szegedi 80 (39), SzNFD (40), Papri King (64), Festival (197), Napfeny (201) and best performer is again Jariza (222).

To consider all traits collectively a weighted analysis is used. This weighting helps identify cultivars for further testing and for combining ability analysis. The results of this weighting are represented in Figure 5 (below).

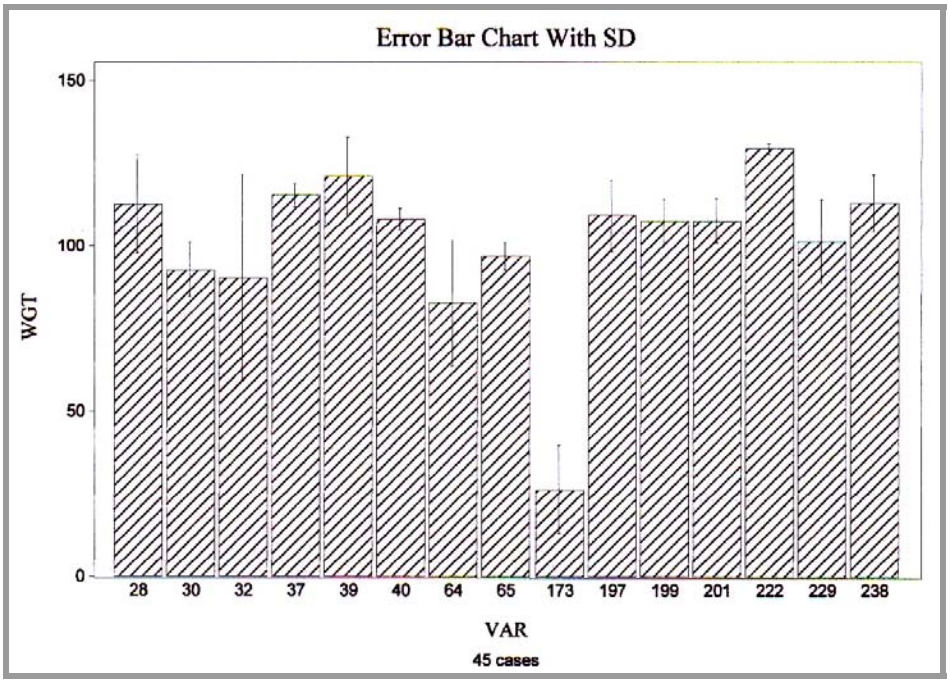


Fig. 5: **Weighted analyses for the varieties and strains tested**

According to this weighting of all three economically important traits, Jariza (222) is the best choice, however, Szegedi 20 (28), Kalocsai 801, Szegedi 80 (39), SzNFD(40) and Cerise Sweet (238) are not significantly inferior to the Spanish cultivar.

A variety and strain trial (VS3) at Leppington cannot be compared to the Cobbitty trial as it was planted much later and its harvest was consequently out of season. However, a subjective evaluation of these results indicates a similar trend to that observed at Cobbitty.

Variety and strain trial 2005-2006

The variety and strain (VS2) trial in 2005-2006 included 10 lines which were selected as the best performing lines in the previous season. A selection of Sz57-13 which performed very well and three cultivars were also included in this trial. The results are summarized in Table 8 (below).

Table 8: **Variety and strain trial yield and dry matter content, 2005-2006**

Acc. Nr	Pedigree	Pedigree code 04-05	Yield/ plant (kg)	% of riped fruits	Dry matt (%)	ASTA
348	Kalocsai 801 2*/Jalapeno	C98.1.1.1.2.1op2(b).1.1	0.404	65	22	284
349	C97.15/ Szegedi 20	C98.65.1.1.1.1op1(b).1.1	0.607	66	17	299
350	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(a).1.1	0.595	55	21	328
351	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(b).1.1	0.592	62	22	268
352	C97.15/ Szegedi 20	C98.65.1.1.2.2op1(c).1.1	0.681	58	21	277
353	C97.15/ Szegedi 20	C98.65.1.1.1.1.1.1.1	0.496	70	20	336
354	C97.15/ Szegedi 20	C98.65.1.1.1.1.2.1.1	0.562	58	18	300
355	C97.15/ Szegedi 20	C98.65.1.1.1.1.2.2.1	0.609	74	20	301
356	C97.15/ Szegedi 20	C98.65.1.1.1.2.1.2.1	0.626	57	17	272
357	C97.15/ Szegedi 20	C98.65.1.1.3.1.1.1.1	0.495	68	25	294
238	Cerise Sweet		0.570	65	23	268
40	Mauthner paprika (SzNFD)		0.457	66	21	327
39	Sz80		0.517	71	22	303
43	Co57-13.3b		0.637	62	25	259
	Mean		0.563	64.2	20.9	294.2
	LSD (5%)		0.189	8.53	4.07	95.1

Variety and strain trial 2006-2007

Two trials were conducted during 2006-2007 (VS3 and VS1) and the results are summarized in tables 9 & 10 (below).

Table 9: **Variety and strain (VS1) trial yield and dry matter content 2006-2007**

Acc. Nr	Pedigree	Pedigree code 04-05	Yield/plot (kg)	% of ripe fruits	Dry matt (%)	ASTA
358	C98.51.1/Conquist.	C99.207.4.3.1op1(a).2.2.1	10.9	73	20	410
359	C98.51.1/Conquist.	C99.207.4.3.1op1(a).2.2.2	16.1	75	23	377
360	C97.15/Sz80	C99.209.3.1.2op1(a).1.2op1(a)	11.1	85	18	260
361	C97.15/Sz80	C99.209.3.1.2op1(c).1.1.1	7.61	63	20	366
362	Sz20/Papri King	C99.33.3.1.1op2(a).2.1op1(a)	14.1	61	19	337
363	Sz20/Papri King	C99.33.3.1.1op3(b).1.2.1	11.3	63	22	369
364	SzNFD/Papri King	C99.85.1.7.1op4(b).2.2op1(a)	11.57	88	22	354
365	SzNFD/Papri King	C99.85.1.7.1op4(c).1.1.1	11.13	86	23	286
366	SzNFD/Papri King	C99.85.1.7.1op4(c).1.2.1	12.57	87	25	338
367	SzNFD/Papri King	C99.85.2.3.1op2(b).3.1.1	9.78	78	24	349
368	SzNFD/Papri King	C99.85.2.3.1op2(b).3.1op1(a)	9.43	62	23	276
369	SzNFD/Papri King	C99.85.2.3.1op2(c).1.1011(a)	11.4	69	23	348
370	SzNFD/Papri King	C99.85.2.3.1op3(a).1.1.1	11.71	55	24	356
371	SzNFD/Papri King	C99.85.2.3.1op3(a).1.1op1(a)	12.48	67	22	381
372	SzNFD/Papri King	C99.85.2.5.2op1(a).1.1op1(a)	11.24	93	25	327
238	Cerise sweet		8.8	73	27	297
229	Sunired		9.6	70	18	349
355	C97.15/ Szegedi 20	C98.65.1.1.1.1.2.2	9.16	84	18	389
	Mean		11.12	74.2	22.2	343.4
	LSD (5%)		6.71	26.1	4.1	72.1

Table 10: **Variety and strain (VS3) trial yield and dry matter content 2006-2007**

Acc.#	Pedigree	Pedigree code	Yield/plant (kg)	% of ripe fruits	Dry matt (%)	ASTA
349	C97.15/ Szegedi 20	C98.65.1.1.1.1op1(b).1.1	11.8	83	20	288
353	C97.15/ Szegedi 20	C98.65.1.1.1.1.1.1.1	12.2	80	20	317
355	C97.15/ Szegedi 20	C98.65.1.1.1.1.2.2.1	11.9	48	19	320
357	C97.15/ Szegedi 20	C98.65.1.1.3.1.1.1.1	14.2	77	21	304
235	Co57-13.3b		16.9	78	20	292
39	Sz80		13.6	73	21	332
40	Mauthner paprika		13.8	73	22	291
238	Cerise Sweet		12.8	67	22	285
	Mean		13.4	72.5	20.6	302.3
	LSD (5%)		4.17	14.2	1.69	41.9

Combining ability test

A small scale combining ability test was conducted in the field during growing seasons as well. Based on observations, the following male and female combinations produced the best F1 hybrid seed:

1. Female parents: Cerise Sweet, Sunired (Uni.of Sydney), Szegedi 80, Kalocsai 801, Szegedi 20, SzNFD (Hungarian), Jaranda, and Jariza (Spanish) cultivars
2. Male parents: Papri King, Papri Queen (USA) and the two Spanish varieties Jariza and Jaranda.

However, other traits such as dry matter, pigment content and machine harvestability must also be considered.

*Fig.*6: **Hybrid paprika plant**

Lines submitted for PBR

Of the lines submitted for Plant Breeders Rights (PBR) registration during the previous project (Sunired, Earlysuni, and Cerise Sweet), Cerise Sweet was accepted (see appendix 1) for PBR.

The cultivars that were used to benchmark Cerise Sweet, Sunired and Earlysuni are shown in table 11 (below). These paprika lines have been used as common standards. The uniformity and stability trial also included the parents that these lines originated.

Table 11: **Field evaluation of PBR trial 2004-2005**

Nr	Name	Parent /candidate	Score
1	Cerise Sweet generation 1	Candidate	6.3
2	Cerese Sweet generation 2		6.3
3	Szentesi NFD	Parent	7.3
4	Sunired generation 1	Candidate	6.5
5	Sunired generation 2		6.5
6	Kalocsai 801	Parent	6.3
7	Earlysuni generation 1	Candidate	5.7
8	Earlysuni generation 2		6.8
9	Szegedi 20	Parent	7.5

The purpose of the PBR trial was to evaluate the stability and uniformity of the potential candidates.

Phenotypic observations were made for PBR purposes (see Cerise Sweet publication from the PBR journal appended).

Six to eight plants per cultivar were selfed for pure seed production.

Fig. 7: **Stability and Uniformity trial for PBR**

Semi-commercial trial

Two semi-commercial trials were conducted of Cerise Sweet and Sunired during 2004-2005 in Victoria. One of the trials was undertaken to increase Cerise Sweet and Sunired for oleoresin extraction (aromatic resin used as a food additive), and the other to increase seed of Cerise Sweet for commercial planting.

Our industry partner, John Vella of Berwick Speedy Seedlings Pty Ltd, conducted these trials on a tomato farm. He has a major client base in Australia and was looking at Paprika and Capsicums as an alternative crop for his tomato growers. The tomato crop has similar agronomic requirements and the tomato mechanical harvesting equipment could be used to harvest paprika if the fruit pickers are adapted to handle the smaller paprika fruits. Tomato growers also use dryers to produce dried tomatoes and it is thought they could also be used to dry paprika fruits.

Seed of the two cultivars was provided to Berwick Speedy Seedlings to grow seedlings for this trial. Six week old seedlings were transplanted by machine. The Cerise Sweet seed increase trial was of limited use because of heavy weed infection and some animal damage. However, the paprika oleoresin production trial was very successful. Digital images (Figure 8) and samples for ASTA testing have been collected.

Figure 8:.**Paprika oleoresin trial on the tomato grower's farm in Victoria**

Seedlings were transplanted three rows per bed, forty centimetres between rows and thirty centimetres between plants. The University of Sydney assisted John Vella in the management of the crop and provided advice when necessary. The crop was harvested by hand when more than 80% of fruits were fully matured.

Samples were collected randomly in the trial for dry matter and ASTA testing.

The fruits were leathery and uniformly ripe at harvesting time (Figure 8).
Table 12 (below) shows the ASTA results and the amount of paprika produced in this trial. A small trial of Cerise Sweet was also conducted to produce organic paprika.

Fig 9: **Harvested fruit of Cerise Sweet**

All harvested fruits were washed, dried and milled and samples were taken for laboratory analyses. The milled product is in freezer storage (-20 degrees).

Table 12: **ASTA results of paprika produced in Victoria during 2004-2005**

Milled paprika	Amount (kg)	ASTA
Cerise Sweet	26	331
Sunired	17	264
Mixed (C.sweet & Sunired)	8	274
Organic	5	324

Single seed descent material

Cooperation with the Szeged Paprika Research Station in Hungary has allowed the utilization of alternative seasons in Europe and Australia. F1 crosses received from Hungary were planted in the field (one row plot) and 2-3 plants were selfed and harvested for pure F2 seed. Many of the hybrids exhibited mechanical harvestability traits. All individually harvested plants were tested for ASTA. Based on pigment content and other characters the best performing populations were selected for the next generation using single seed descent (SSD). The SSD method was employed to advance generations quickly and economically, and has proven to be very effective. SSD involved growing plants in very small amounts of potting media in seedling trays until maturity in microclimates at the PBI Cobbitty. These sowings were conducted throughout the year to quickly advance generations and trait fixation. During SSD, selection was limited to the elimination of plants with erect fruit, and the selection of plants with apparent high pigment content based on fruit colour. In the 2003-2004, F2 and F3 generations were grown in the off season, which made it possible to transplant the F4 generation in late October.

Fig. 10: **Growth of SSD material in a microclimate environment**

The populations in Table 13 were sown as an F2 generation in September 2005 and advanced using SSD. These populations were derived from F1s harvested in 2004-2005.

Table 13: **F2 generation advanced using SSD in 2005-2006**

Plot Nr	Pedigree	Pedigree code
1	Sunired/Bambino	CO4.1.b
2	Sunired/Papri King	CO4.2.b
3	Super shepherd/P.King	CO4.66.b
4	Bambino/Sunired	CO4.67.b
5	Jaranda/SudAfrika	CO4.70.b
6	Jariza/P.mild	CO4.72.b
7	Jariza/Jaranda	CO4.79.b
8	Jariza/SudAfrika	CO4.85.b
9	P. Mild/Jariza	CO4.89.b
10	P.Mild/Jaranda	CO4.92.b
11	P.Mild/SudAfrika	CO4.95.b
12	SudAfrika/Jariza	CO4.98.b
13	SudAfrika/Jaranda	CO4.99.b
14	SudAfrika/Pmild	CO4.101.b

The single plant progenies carried forward using the SSD system, including the F5 materials (table14), were harvested and are stored in the long term seed storage facility at PBI Cobbitty.

Table 14: **Harvested F5 embryos derived from SSD**

Plot Nr	Pedigree	Pedigree Code
301	Sz57-13/Jaranda	CO2.1.1.b.b.b
302	202/205	CO2.89.1.b.b.b
303	Sz57-13/Jaranda	CO2.3.1.b.b.b
304	1/4-2/205	CO2.93.1.b.b.b
305	Sz57-13/Jariza	CO2.8.1.b.b.b
306	Sz57-13/Jariza	CO2.9.1.b.b.b
307	Sz57-13/Sud Africa	CO2.10.1.b.b.b
308	Sz57-13/Sud Africa	CO2.12.1.b.b.b
309	Sz178/Jaranda	CO2.13.1.b.b.b
310	Sz178/Jaranda	CO2.14.1.b.b.b
311	Szentes NFD/ NuMex Primavera	CO2.16.1.b.b.b
312	K57-231/Jariza	CO2.23.1.b.b.b
313	K801.2/ NuMex Primavera	CO2.24.1.b.b.b
314	K801.2/ NuMex Primavera	CO2.25.1.b.b.b
315	K801.2/ NuMex Primavera	CO2.26.1.b.b.b
316	Jaranda/Sz80	CO2.29.1.b.b.b
317	Jariza/Sz80	CO2.37.1.b.b.b
318	Corno di Toro/Sz80	CO2.46.1.b.b.b
319	Corno di Toro/NuMex Primavera	CO2.47.1.b.b.b
320	Corno di Toro/K801.2	CO2.51.1.b.b.b
321	Papri Mild c/Sz57-13	CO2.53.1.b.b.b
322	Papri Mild c/K801.2	CO2.53.2.b.b.b
323	Papri Mild c/K801.2	CO2.55.1.b.b.b
324	Papri Mild c/Sz80	CO2.57.1.b.b.b
325	Papri Mild c/Sz80	CO2.59.1.b.b.b
326	NuMex Primavera/Sz20	CO2.60.1.b.b.b
327	NuMex Primavera/Sz57-13	CO2.62.1.b.b.b
328	NuMex Primavera/Sz80	CO2.64.1.b.b.b
329	NuMex Primavera/Sz80	CO2.65.1.b.b.b
330	Del-Africa/Sz57-13	CO2.68.1.b.b.b
331	Papri King/NuMex Primavera	CO2.70.1.b.b.b

Advanced lines progressed in the field

Advanced lines derived from SSD technique were planted in the field for further investigation and selections. Table 15 shows the F6 lines included in the advanced lines trial during 2005-2006. Based on field selections and laboratory analyses a number of F6 lines performed well. The 15 highest performing lines were selected and included in the 2006/07 VS1 trial, as described earlier on this report.

Table 15: **F6 generations 2005-2006**

Pedigree	Pedigree Code	Detachability	Fruits/plant	Dry matter.%	ASTA
C98.51.1/Conquist.	C99.207.4.3.1op1(a).2.2.1	Dett.	66	18	363
C98.51.1/Conquist.	C99.207.4.3.1op1(a).2.2.2	Dett.	39	17	328
C98.51.1/Conquist.	C99.207.4.3.1op1(a).2.2op1(a)	Dett.	54	16	327
C97.15/Sz80	C99.209.3.1.2op1(a).1.1.1	Dett.	106	17	138
C97.15/Sz80	C99.209.3.1.2op1(a).1.1.2	Dett.	51	17	196
C97.15/Sz80	C99.209.3.1.2op1(a).1.2.1	Dett.	67	17	214
C97.15/Sz80	C99.209.3.1.2op1(a).1.2op1(a)	Dett.	87	18	278
C97.15/Sz80	C99.209.3.1.2op1(c).1.1.1	Dett.	84	22	246
Sz20/Papri King	C99.33.3.1.1op2(a).2.1op1(a)	semidett	44	22	279
Sz20/Papri King	C99.33.3.1.1op3(b).1.2.1	semidett	53	22	376
Sz20/PM 1231	C99.40.1.2.2op2(a).1.1.1	Dett.	63	22	221
SzNFD/Papri King	C99.85.1.7.1op2(c).2.1.1	Dett.	38	15	294
SzNFD/Papri King	C99.85.1.7.1op2(c).2.1.2	Dett.	24	22	211
SzNFD/Papri King	C99.85.1.7.1op4(b).2.2op1(a)	Dett.	86	22	388
SzNFD/Papri King	C99.85.1.7.1op4(c).1.1.1	Dett.	79	22	232
SzNFD/Papri King	C99.85.1.7.1op4(c).1.2.1	Dett.	35	23	360
SzNFD/Papri King	C99.85.2.3.1op2(a).1.2.1	Dett.	64	16	361
SzNFD/Papri King	C99.85.2.3.1op2(a).2.1.1	Dett.	56	17	386
SzNFD/Papri King	C99.85.2.3.1op2(a).2.1op1(a)	Dett.	84	16	361
SzNFD/Papri King	C99.85.2.3.1op2(b).3.1.1	Dett.	54	20	312
SzNFD/Papri King	C99.85.2.3.1op2(b).3.1op1(a)	Dett.	69	18	361
SzNFD/Papri King	C99.85.2.3.1op2(c).1.1.1	Dett.	26	17	207
SzNFD/Papri King	C99.85.2.3.1op2(c).1.1011(a)	Dett.	67	19	358
SzNFD/Papri King	C99.85.2.3.1op3(a).1.1.1	Dett.	37	15	236
SzNFD/Papri King	C99.85.2.3.1op3(a).1.1op1(a)	Dett.	87	17	361
SzNFD/Papri King	C99.85.2.5.2op1(a).1.1op1(a)	Dett.	60	23	238

ASTA method

Selection for high pigment content was a key criterion in the breeding program (Table 6), and a method for analysis of this parameter was developed. Extractable colour was measured in units of ASTA according to the ASTA official analytical method 20.1 (ASTA, 1997). Four representative sub-samples of each ground sample (70-100 mg) were weighed and transferred to a 100 ml volumetric flask. Acetone (100ml) was added. The flasks were shaken and kept in the dark for at least 16 hours at room temperature. Portions of each of the sample extracts were then transferred to 96 well plates, or to cuvettes of a spectrophotometer. The absorption was recorded at 460 nm with a ZEISS PM2A spectrophotometer, calibrated with an acetone blank, or with a similarly calibrated 96 well plate reader.

ASTA colour units were calculated using the following formula:

$$ASTA\ color = \frac{Absorbance_{460nm}\ of\ the\ sample\ extract \times 16.4 \times If}{Sample\ weight\ (g)}$$

In this formula *If* is the instrument correction factor which is calculated by dividing the declared absorbance of a glass reference standard by absorbance obtained at 465 nm on a glass reference standard.

The ASTA method was performed at PBI Cobbitty, and at the NATA-registered laboratory of BRI Pty Ltd, North Ryde, where the exact ASTA official analytic method 20.1 was used. This allowed us to compare our results with an accredited laboratory. Two minor variants of our method using 96 well plates were tested; plates were measured with either their own perspex lid, or with a cling wrap seal; followed by evaluation using a PM2A spectrophotometer.

Paprika production

Milling of harvested and dried paprika was conducted at three levels; samples from cultivar trials, larger quantities from more advanced material and large scale test-milling. The test milling resulted in very highly milled paprika. Test milling indicated that when a medium screen was used the milled product was not fine enough. The product was sifted and what did not pass through a 0.3 mm screen was then milled again. Products with a smaller particle size gave higher pigment extraction. All cultivars samples were sent to BRI Pty Ltd for pigment testing.

Oleoresin for smallgoods manufacturing and spice production requires extract from high quality paprika, preferably Hungarian or Basque fruit types; taste and bouquet is extremely important. The price is dependent on ASTA values, which could range from 1000-4000 ASTA. The ASTA values are adjusted after extraction by diluting the extract with various vegetable oils. The oleoresin extraction process automatically sterilises the product. All milled products were sanitised by ionising radiation using a dose of 10 kGy of gamma radiation. Following this, another ASTA test was conducted on all irradiated milled paprika.

Capsicum hybrid seed production

The objective of this component of the project was to develop a system for producing hybrid seed of paprika *(Capsicum annuum var. annuum* Longum Group) in commercial quantities at an affordable cost in Australia. The production of hybrid seed in capsicum is time- and labour- intensive, and therefore expensive, but more than justified by hybrid vigour for yield. Various aspects of hybrid seed production based on male sterility were investigated.

Male sterile lines

The F1 and F2 generation genetic male sterile (ms) paprika lines used in this project are listed in Table 16. Emergence date and hypocotyl colour were recorded. The F1-generation is underlined and the F2-generation is in normal font.

At flowering time the segregation of the F2 population was recorded based on anther morphology and size. The suspected ms plants were marked and pollen testing was undertaken for each marked plant. Pollen was stained and also tested for germination ability.

Table 16: **Male sterile genetic material sown on 8/05/06**

My Code	Pedigree Code	Pedigree	Special notes	Emerg date	Hypoc. color	Nr.plants transpl.
A30	CH03.1.1	HM3/Papri Queen	1 tray	19/05	Sgr	55
A31	CH03.1.2	HM3/Papri Queen	"	19/05	Sgr	60
A32	CH03.6.1	HM3/Cerise Sweet	"	19/05	Sgr	55
A33	CH03.6.2	HM3/Cerise Sweet	"	20/05	Sgr	55
A34	CH03.7.1	HM3/Conquistador	"	19/05	Sgr	60
A35	CH03.7.2	HM3/Conquistador	"	19/05	Sgr	50
A36	CH03.9.1	HM2/Papri Queen	"	20/05	Sgr	60
A37	CH03.11.1	HM2/Sunired	"	20/05	Sgr	55
A38	CH03.12.1	HM3/Sunired	"	20/05	Sgr	55
A21	CH03.7	HM3/Conquistador	1 punn.	19/05		7
A22	CH03.1	HM3/Papri Queen	"	19/05		7
A23	CH03.17	HM3/Sunired	"		Not germ.	
A24	CH03.14	HM3/Cerise Sweet	"	21/05		8

Transplanting date: 3-4 of July in 10 cm pots in the greenhouse

Pollen staining test

Fresh flowers were collected at the time of anther dehiscence and in small Petri dishes and carried to the laboratory. Anthers were placed on a microscope slide and carefully opened. A drop of acetone carmine was added and a cover slip was placed over the anthers, 5 minutes later, once the solution entered the cells, pollen grains were examined under the microscope.

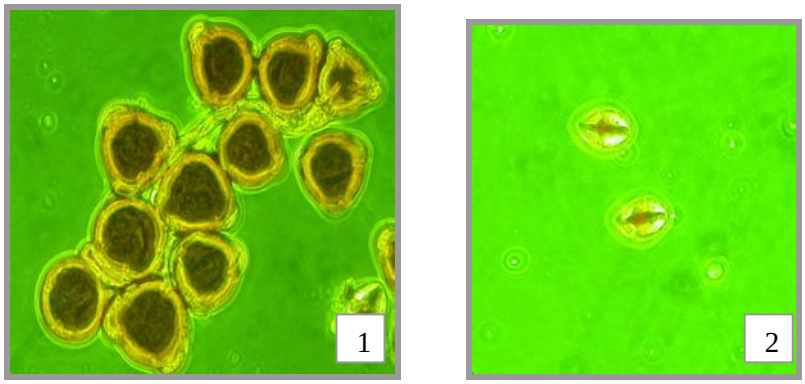
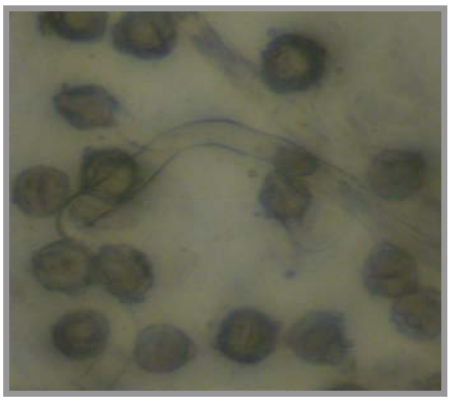


Fig. 11: **Male fertile (1) and male sterile (2) plant**

Viable pollen grains are stained and have a triangular shape; non-viable grains are unstained and round in shape. It was difficult to locate any pollen grains in the ms plants and no viable pollen grains were found in these plants.

Pollen germination test

Pollen was sprinkled on a medium (6% agar, 3% sucrose, 0.01% calcium chloride) prepared in Petri dishes. The Petri dishes were kept at constant room temperature (20-22ºC) for 24 hours and pollen tube growth was subsequently observed under the microscope.



Pollen grains that developed a normal pollen tube were considered fertile and those that did not develop a normal pollen tube were considered unviable. The confirmed ms plants were then used as the female parent to initiate further crosses and the F1 plants (listed in table 16) were used as the pollen source. Two to three fruit of the male fertile plants in the F2 were harvested for seed extraction.

Fig.12: **Pollen germination observed under microscope**

Visual evaluation of ms plants was corroborated100% by the pollen staining and germination, indicating that for practical breeding, a visual evaluation is all that is required (Figure 13).

Fig.13: **Visual evaluation of male sterile flowers segregating in the F2 generation**

As the research progressed, two issues became important; how to increase the frequency of ms plants in a given population and how to propagate or maintain these ms materials.

The genetic ms used in the project is the recessive ms3 gene which will give a maximum of 50% ms in any given population. To achieve a 50% frequency of ms individuals, ms plants in the F2 must be crossed with plants carrying the recessive gene. However, this approach was flawed as one third of the population is expected to carry the recessive gene while the remainder are fully fertile. To avoid this, F1 plants from the original cross, all carrying the recessive ms3 gene, where crossed to the ms F2 plants (table 17).

F1/F2	A21	A22	A24
A30		X	
A31		X	
A32			X
A33			X
A34	X		
A35	X		
A36		X	
A37			X
A38			X

Table 17: **Intermediate F2 x F1 crosses**

Table 18: **List of successful crosses from male sterile material 2006**

Cross Nr.	Female	Male	Pedigree	Nr.of seeds
165	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	20
47	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	20
73	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	30
70	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	20
40	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	15
14	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	50
13	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	30
11	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	35
8	A31	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	55
61	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	40
57	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	40
87	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	35
85	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	50
58	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	30
29	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	30
28	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	25
27	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	40
25	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	20
23	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	25
21	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	6
20	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	20
19	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	45
17	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	35
60	A30	A22	(HM3/Papri Queen)/(HM3/Papri Queen)	45
163	A33	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	10
164	A33	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	14
215	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	35
204	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	6
197	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	10
158	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	14
160	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	12
211	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	20
161	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	20
162	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	25
157	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	10
54	A32	A24	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)	15
130	A38	A24	(HM3/Sunired)/(HM3/Cerise sweet)	22
134	A38	A24	(HM3/Sunired)/(HM3/Cerise sweet)	25
138	A38	A24	(HM3/Sunired)/(HM3/Cerise sweet)	35
141	A38	A24	(HM3/Sunired)/(HM3/Cerise sweet)	30
140	A38	A24	(HM3/Sunired)/(HM3/Cerise sweet)	50
104	A38	A24	(HM3/Sunired)/(HM3/Cerise sweet)	25
93	A36	A22	(HM2/Papri Queen)/(HM3/Papri Queen)	30
125	A37	A24	(HM2/Sunired)/(HM3/Cerise Sweet)	35
126	A37	A24	(HM2/Sunired)/(HM3/Cerise Sweet)	45
142	A37	A24	(HM2/Sunired)/(HM3/Cerise Sweet)	40
132	A37	A24	(HM2/Sunired)/(HM3/Cerise Sweet)	20
133	A37	A24	(HM2/Sunired)/(HM3/Cerise Sweet)	10
128	A37	A24	(HM2/Sunired)/(HM3/Cerise Sweet)	12
193	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	25
149	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	10
119	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	25
150	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	20
174	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	35
191	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	13
173	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	55
123	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	55
122	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	25
121	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	40
115	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	15
113	A34	A21	(HM3/Conquistador)/(HM3/Conquistador)	10
79	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	50
7	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	45
6	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	40
5	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	50
181	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	13
175	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	50
186	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	10
185	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	30
168	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	30
167	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	35
187	A35	A21	(HM3/Conquistador)/(HM3/Conquistador)	20

Table 19: **Segregation of F2 male sterile population derived from the intermediate**

Nr	Pedigree		Nr of plants sown (F1s)	F2 segregation			
	Female F2 (msms)	Male F1(Msms)		ms	Fert	ms %	Fert %
1	(HM3/Papri Queen)/(HM3/Papri Queen)		9	4	5	44	56
2	(HM3/Papri Queen)/(HM3/Papri Queen)		10	5	4	50	40
3	(HM3/Papri Queen)/(HM3/Papri Queen)		11	4	5	36	45
4	(HM3/Papri Queen)/(HM3/Papri Queen)		12	3	3	25	25
5	(HM3/Papri Queen)/(HM3/Papri Queen)		12	5	6	42	50
6	(HM3/Papri Queen)/(HM3/Papri Queen)		12	4	5	33	42
7	(HM3/Papri Queen)/(HM3/Papri Queen)		12	6	6	50	50
8	(HM3/Papri Queen)/(HM3/Papri Queen)		12	5	6	42	50
9	(HM3/Papri Queen)/(HM3/Papri Queen)		11	1	3	9	27
10	(HM3/Papri Queen)/(HM3/Papri Queen)		12	4	4	33	33
11	(HM3/Papri Queen)/(HM3/Papri Queen)		12	2	2	17	17
12	(HM3/Papri Queen)/(HM3/Papri Queen)		10	3	3	30	30
13	(HM3/Papri Queen)/(HM3/Papri Queen)		11	3	3	27	27
14	(HM3/Papri Queen)/(HM3/Papri Queen)		12	3	4	25	33
15	(HM3/Papri Queen)/(HM3/Papri Queen)		12	4	4	33	33
17	(HM3/Papri Queen)/(HM3/Papri Queen)		10	5	5	50	50
18	(HM3/Papri Queen)/(HM3/Papri Queen)		9	4	4	44	44
19	(HM3/Papri Queen)/(HM3/Papri Queen)		13	3	3	23	23
20	(HM3/Papri Queen)/(HM3/Papri Queen)		13	3	3	23	23
21	(HM3/Papri Queen)/(HM3/Papri Queen)		12	4	4	33	33
22	(HM3/Papri Queen)/(HM3/Papri Queen)		12	4	6	33	50
23	(HM3/Papri Queen)/(HM3/Papri Queen)		9	4	4	44	44
24	(HM3/Papri Queen)/(HM3/Papri Queen)		11	3	3	27	27
25	(HM3/Papri Queen)/(HM3/Papri Queen)		12	2	2	17	17
26	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	6	5	50	42
27	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		8	3	3	38	38
28	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	5	4	42	33
29	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	7	5	58	42
30	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	4	4	33	33
31	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		9	4	4	44	44
32	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		11	4	3	36	27
33	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		6	4	4	67	67
34	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		11	1	2	9	18
35	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	4	3	33	25
36	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	5	4	42	33
37	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	4	6	33	50
38	(HM3/Cerise Sweet)/(HM3/Cerise Sweet)		12	6	6	50	50
39	(HM3/Sunired)/(HM3/Cerise sweet)		11	2	2	18	18
40	(HM3/Sunired)/(HM3/Cerise sweet)		8	2	3	25	38
41	(HM3/Sunired)/(HM3/Cerise sweet)		12	2	2	17	17
42	(HM3/Sunired)/(HM3/Cerise sweet)		14	3	3	21	21
43	(HM3/Sunired)/(HM3/Cerise sweet)		12	4	3	33	25
44	(HM3/Sunired)/(HM3/Cerise sweet)		12	2	4	17	33
45	(HM2/Papri Queen)/(HM3/Papri Queen)		10	5	4	42	33
46	(HM2/Papri Queen)/(HM3/Papri Queen)		12	3	3	25	25
47	(HM2/Papri Queen)/(HM3/Papri Queen)		12	5	4	42	33
48	(HM2/Sunired)/(HM3/Cerise Sweet)		11	6	5	55	45
49	(HM2/Sunired)/(HM3/Cerise Sweet)		12	3	3	25	25
50	(HM2/Sunired)/(HM3/Cerise Sweet)		11	6	5	55	45
51	(HM2/Sunired)/(HM3/Cerise Sweet)		10	4	3	40	30
52	(HM2/Sunired)/(HM3/Cerise Sweet)		10	3	2	30	20
53	(HM2/Sunired)/(HM3/Cerise Sweet)		9	2	4	22	44
54	(HM3/Conquistador)/(HM3/Conquistador)		11	5	5	45	45
55	(HM3/Conquistador)/(HM3/Conquistador)		10	3	2	30	20
56	(HM3/Conquistador)/(HM3/Conquistador)		12	5	2	42	17
57	(HM3/Conquistador)/(HM3/Conquistador)		10	4	4	40	40
58	(HM3/Conquistador)/(HM3/Conquistador)		12	3	3	25	25
59	(HM3/Conquistador)/(HM3/Conquistador)		7	1	0	14	0
60	(HM3/Conquistador)/(HM3/Conquistador)		10	4	2	40	20
61	(HM3/Conquistador)/(HM3/Conquistador)		12	3	3	25	25
62	(HM3/Conquistador)/(HM3/Conquistador)		13	2	2	15	15
63	(HM3/Conquistador)/(HM3/Conquistador)		12	1	1	8	8
64	(HM3/Conquistador)/(HM3/Conquistador)		11	1	1	9	9

Nr	Pedigree		Nr of plants sown (F1s)	F2 segregation			
	Female F2 (msms)	Male F1(Msms)		ms	Fert	ms %	Fert %
66	(HM3/Conquistador)/(HM3/Conquistador)		12	4	3	33	25
67	(HM3/Conquistador)/(HM3/Conquistador)		12	4	3	33	25
68	(HM3/Conquistador)/(HM3/Conquistador)		12	4	4	33	33
69	(HM3/Conquistador)/(HM3/Conquistador)		12	5	6	42	50
70	(HM3/Conquistador)/(HM3/Conquistador)		12	6	6	50	50
71	(HM3/Conquistador)/(HM3/Conquistador)		12	4	6	33	50
72	(HM3/Conquistador)/(HM3/Conquistador)		12	6	4	50	33
73	(HM3/Conquistador)/(HM3/Conquistador)		12	4	4	33	33
74	(HM3/Conquistador)/(HM3/Conquistador)		11	4	4	36	36
75	(HM3/Conquistador)/(HM3/Conquistador)		12	4	4	33	33
76	(HM3/Conquistador)/(HM3/Conquistador)		11	1	1	9	9
77	(HM3/Conquistador)/(HM3/Conquistador)		9	3	4	33	44
78	(HM3/Conquistador)/(HM3/Conquistador)		9	3	3	33	33

SDS PAGE for protein identification

An attempt was made to identify the presence of the ms gene based on proteins using the protein separation method of sodium dodectyl sulfate polyacrylamide gel electrophorensis (SDS-PAGE). As the equipment used was very old and the time was limited, not all the lines were tested using this method. Nevertheless, it was successful in identifying ms lines in the seedling stage.

Fig. 14: **SDS PAGE for protein identification on ms paprika lines**

Vegetative production of male sterile lines

The propagation of male sterile individuals was investigated. Cuttings were taken of ms plants and various media were used to encourage root growth. Quite promising results have been achieved and the production cost has been estimated (table 21). Vegetative propagation of genetic ms lines provides a low cost alternative for hybrid seed production, bypassing the need for phenotype identification, progeny testing and/or marker screening. Moreover, vegetative propagation can also be cost effective of maintaining cytoplasmic sterile lines. Male sterile capsicum lines were successfully propagated during this study as cuttings in the greenhouse. A preliminary trial was conducted to investigate rooting percentage using different growth media (Jiffy plugs, OASIS cubes, seedling mixture plus vermiculite or perlite). After a period of 4-5 weeks in a misting chamber 100% rooting was achieved in a media consisting of 50% seedling mixture / 50% vermiculite.

Fig. 15: **Cuttings in 50% vermiculite & 50% seedling mix in the misting cabinet**

This experiment was repeated twice with a single Ethrel® (ethephon) application (250 ppm) to delay flowering and to induce vegetative growth. An attempt was made to propagate ms lines in vitro. As time and resources were limited it was not possible to pursue this further. Table 22 compares the cost of producing 1kg of hybrid seed using traditional hand pollination, our technique of producing male sterile lines using vegetative propagation and pollination by native bees, with the cost of purchasing 1kg of hybrid seed from international providers. These comparisons are estimates only and need to be verified. However, the use of vegetatively propagated male sterile lines to produce F1 hybrid seed is clearly more cost effective than traditional avenues of producing or obtaining hybrid seed.

Table 21: **Production cost for vegetative propagation by cuttings**

No.	Items	Cost/1000 ($AU)
1	Mother plants maintenance cost ##	300
2	Collection and planting charge*	111.75
3	Cost of container**	35.2
4	Cost of potting mixture**	3.26
5	Plant material maintenance charge*	17.25
6	Vermiculite#	7
7	Cost of misting room (capital + maintenance)***	95
	Total	569.46

**hourly pay rate = $AU 15*
***OASIS prices; potting mix/cubic meter = $AU 108.63; punnets 6'S/ unit = $AU 0.12;trays/unit = $ AU 0.80*
****capital cost = $AU 400/year; maintenance cost = $AU 59.5/month*
 # Elders Pty Ltd price / 100L bag = $ AU 23.24
maintenance of mother plants (sowing, raising cost)
Average number of cuttings/plant = 30.5

Table 22: **Capsicum seed cost for seed based production; cuttings based production and imported seeds**

	Seed based production	**Cutting based production**	**Imported seed**
Heterozygote generation	$68[1]	na	na
Male sterile plant generation	$709[2]	$620[3]	na
Male fertile plant generation	$428[4]	$214[5]	
Pollination	$5000	$ 0	
Transplant costs	$320[6]	$160[7]	na
Male fertile removal	$360[8]	na	na
Fruit and seed harvest	$3000[9]	$3000[9]	na
Expected yield	414,640 seeds=3.110kg	414,640 seeds=3.110kg	
Cost per kg hybrid seed (AUD)	$6571	$1284	$13800-$36700[10]

[1] Estimated cost to generate the heterozygous (MS/ms) male parent of the population that will segregate for male sterility. Labour, glasshouse and materials costs are included.

[2] Estimated cost to hybridise heterozygotes generated in item [1] with male sterile homozygotes.

Seventy hybridisations between male fertile heterozygotes and male sterile homozygotes is expected to generate approximately 2100 seeds. This cost estimate consists of labour, glasshouse space for 20 parent plants (4 male fertile heterozygotes and 16 plants from a segregating population- containing approximately 8 male sterile plants), and associated media and pots costs of germination and growing them to maturity.
Costs of harvesting 70 fruits, and extracting and germinating approximately 2000 seeds is considered within this component. Expenses of associated labour, glasshouse space, seedling trays and media are considered here.

[3] Estimated cost to maintain 33 male sterile stock plants, and harvesting and striking 1000 cuttings. Labour, glasshouse and materials charges are considered.

[4] Estimated cost to grow and self pollinate male fertile plants to generate 1000 seed
This cost consists of labour, glasshouse costs to house 4 parent plants, and associated media and pots costs of germination and growing to maturity.
Costs of harvesting 35 fruits, and extracting and germinating approximately 1000 seeds is considered within this component. Expenses of associated labour, glasshouse space, seedling trays and media are considered here

[5] Estimated costs to self pollinate male fertile plants to generate 500 seed.
This cost consists of labour, glasshouse space for 2 parent plants, and associated costs of germination and growing on to maturity.
Costs of harvesting 35 fruits, and extracting and germinating approximately 500 seeds is considered within this component. Expenses of associated labour, glasshouse space, seedling trays and media are also considered here

[6] Estimated cost of raising 2000 seedlings of a) a segregating population (to generate approximately 1000 male sterile plants of female parent) and b) 1000 male fertile seedlings (male parents) and transplanting them to the field. This cost includes labour (16 hours) but does not include land expenses (1000m2) or consider capital, maintenance and operating costs associated with transplanters.

[7] Estimated cost of transplanting a) 1000 cuttings of male sterile plants and b) 500 male fertile seedlings (male parents) to the field. This cost does not include land expenses (1000m2) or consider capital, maintenance and operating costs associated with transplanters.

[8] Estimated labour cost of removal of male fertile plants from female parent rows.

[9] Estimated cost of mechanical harvesting of fruit and isolation of 3.11kg (415,000 with 7.5g 1000 seed wt) of seed from 1000 male sterile plants.

[10] Prices are featured in the November 2006 catalogue of Fairbanks Seeds, West Melbourne, Vic 3003 and are based on 50000 seed price and a 1000 seed weight of 7.5g.

Discussion

Several promising lines selected from stage 3 of the variety and strain trial (VS3) have been identified for possible Plant Breeders Rights (PBR) protection. The continuation of conventional breeding led to the release of the cultivar "Cerise Sweet" with good paprika characteristics (high dry matter % and high extractable pigment content). This new cultivar, "Cerise Sweet" offers the industry significant advantages and could be promoted to interested growers. A commercial partner will need to be found to undertake the bulking up and commercialisation of "Cerise Sweet" and the submission of other promising lines for PBR testing.

The SSD method worked well, enabling interspecific cross material to progress quickly to field trial. Promising materials with paprika-like quality and excellent detachability were identified.

To establish a working system for the production of F1 hybrid capsicum seed two main issues must be addressed; these are increased frequency of ms plants in a given population and cost effective maintenance of ms stocks. In the first instance, male sterile plants were crossed with fertile plants derived from the same parent combination. In the second instance, ms individuals were propagated by cuttings. The results indicate that the use of vegetatively propagated male sterile lines to produce F1 hybrid seed is clearly more cost effective than traditional avenues of producing or obtaining hybrid seed.

The findings of this study have application beyond paprika; they apply to the whole Capsicum genus, including the vegetable and ornamental sectors.

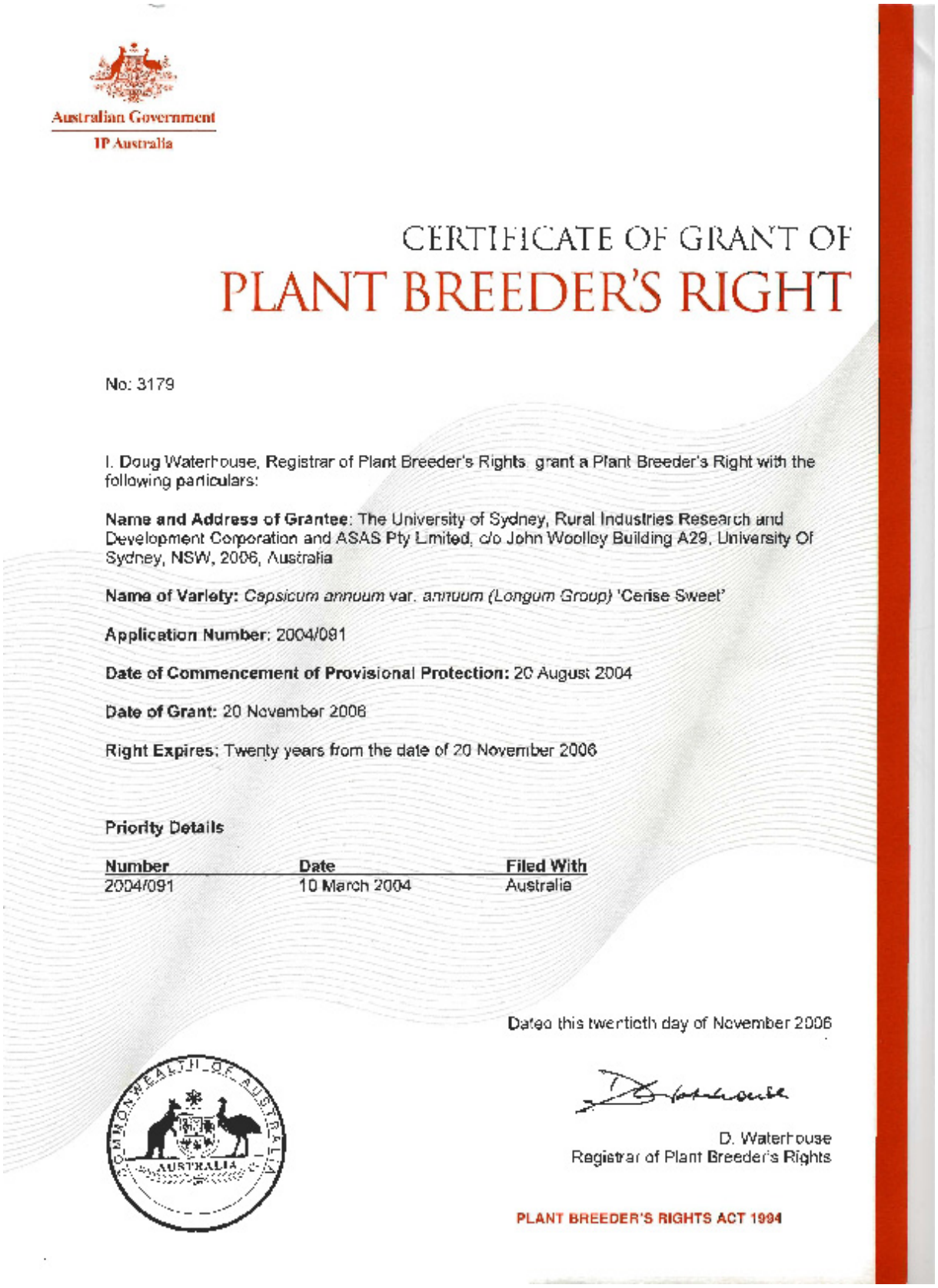
Improved paprika cultivars, paprika germplasm, better breeding and propagation methods arising from this project are available to all stakeholders. The new interspecific materials developed can be advanced and used to breed paprika cultivars, alternatively they may be commercialised following further evaluation. The hybrid seed production results and the vegetative propagation of ms lines provide a basis for the establishment of a commercial hybrid seed production industry in Australia. This would not be restricted to paprika alone, but could be applied across all Capsicum species.

Unfortunately, the paprika market is small in Australia and unlikely to grow significantly. In addition, the infrastructure required to produce dried and crushed condiment paprika for retail sale is expensive and not currently available to most growers. The hybrid F1 seed results are promising and a cost effective F1 hybrid seed production system can be established and applied to the much larger vegetable capsicum industry. However, combining ability and the identification of optimal heterotic combinations are still to be established.

Vegetable capsicum production is an important industry in Australia and hybrid seed is imported at significant cost from overseas suppliers. The findings of this research indicate that hybrid capsicum seed production is likely to be successful in Australia. We recommend that further work be undertaken to examine the transferability of these findings to vegetable capsicum production in Australia.

Finally, it is recommended that an investigation be conducted into the potential market for paprika oleoresin in Australia that could be supplied by Australian producers. This should look into the gaps and obstacles that need to be overcome in creating an efficient and cost effective new rural industry.

Appendices
Cerise Sweet



Australian Government
IP Australia

CERTIFICATE OF GRANT OF
PLANT BREEDER'S RIGHT

No: 3179

I, Doug Waterhouse, Registrar of Plant Breeder's Rights, grant a Plant Breeder's Right with the following particulars:

Name and Address of Grantee: The University of Sydney, Rural Industries Research and Development Corporation and ASAS Pty Limited, c/o John Woolley Building A29, University Of Sydney, NSW, 2006, Australia

Name of Variety: *Capsicum annuum* var. *annuum (Longum Group)* 'Cerise Sweet'

Application Number: 2004/091

Date of Commencement of Provisional Protection: 20 August 2004

Date of Grant: 20 November 2006

Right Expires: Twenty years from the date of 20 November 2006

Priority Details

Number	Date	Filed With
2004/091	10 March 2004	Australia

Dated this twentieth day of November 2006

D. Waterhouse
Registrar of Plant Breeder's Rights

PLANT BREEDER'S RIGHTS ACT 1994

Poster paper


Szegedi 20


Szegedi 80


Szegedi 57-13


Kalocsai 801


Papri Queen


Conquistador

Performance of Some Hungarian and USA Paprika Cultivars Under Australian Conditions

Adriana Hoxha, Prof. N.F. Derera and Prof. P.J.Sharp
The University of Sydney, Plant Breeding Institute
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Introduction

Condiment paprika (*Capsicum annuum* var. *annum* Longum group), has long fruits with intensive red colour, and contains no capsaicin. It is used as a spice in cooking, colouring agent in food manufacture and cosmetic industry and for the extraction of oleoresin. Australia currently imports over 600t of condiment paprika at a cost of over $5 million a year (Sharp, 2004). Since one of our aims is mechanical harvesting, it is therefore important to know what portion of the yield can be removed in one operation. The quality of milled paprika is another aspect to be considered, and this is very much dependant on the time of harvesting and the extractable pigment content. As oleoresin production is another of our objectives, production of high pigment per unit area is very important component.

Capsicum cultivars are successfully grown in different parts of Australia eg. SA, NSW and Queensland (Derera, 2005). As the growth requirements of paprika are very similar to those of capsicum, these areas would be the most appropriate for growing paprika.

Materials and Methods

Four Hungarian (Szegedi 80, Szegedi 20, Szegedi 57-13, Kalocsai 801) and two USA cultivars (Papri Queen and Conquistador) were compared for yield and fruit characters during 2003-2004 and 2004-2005 seasons, using a complete randomised block with 4 replications. Transplanting was done in October (40cm x 30cm), and harvesting in March. Red and green fruits were harvested separately and yield and fruit characters were measured. Extractable colour was measured in ASTA units according to the official analytical method 20.1 (ASTA, 1997). Data were analysed by two way ANOVA.

Results

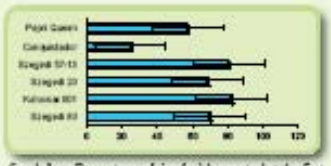
Table 1 shows the average data of two seasons for fruit characteristics and Table 2 shows the average data for yield and its components. Graph 1 shows the percentage of the yield harvested at the first harvesting and Graph 2 shows total fresh yield and half product per unit area.

Table 1: Fruit characteristics
(average data of two seasons)

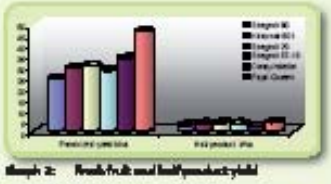
Cultivars	Volume (ml)	Weight (g)	Length (mm)	Width (mm)
Szegedi 80	44.83c	27.21d	109.8d	26.87c
Kalocsai 801	46.25c	29.37d	101.29e	27.98c
Szegedi 20	34.19d	23.94e	100.32e	24.04d
Szegedi 57-13	49.48c	31.84c	125.41c	27.02c
Conquistador	101.45a	66.33a	160.08a	38.44a
Papri Queen	82.54b	42.55b	150.26b	34.96b

Table 2: Yield and its components
(average data of two seasons)

Cultivar	Yield at 1st harvest (t/ha)	Dry matter content (DW%)	Extractable colour (ASTA)	Total pigment (kg/ha)
Szegedi 80	17.64c	16.95bc	326.25a	30.54b
Kalocsai 801	24.04ab	16.03cd	201.12b	24.59b
Szegedi 20	21.38bc	19.32a	323.63a	39.25a
Szegedi 57-13	22.57abc	17.07bc	196.87b	23.66b
Conquistador	8.8d	15.01d	161.75c	12.42c
Papri Queen	27.35a	17.64b	208.12b	26.16b



Graph 1: Percentage of ripe fruit harvested at the first harvest


Graph 2: Fresh fruit and half product yield

Discussion

A synchronised ripening is an important factor for mechanical harvesting where a large portion of fruits can be removed in one operation. USA cultivars displayed late maturity comparatively with the Hungarian ones (Graph 1), which makes it difficult to use one operation harvesting systems while for Kalocsai 801 and Szegedi 57-13 over 80% of the fruits was harvest in one operation. The USA cultivars produced larger fruits than the Hungarian ones, where Conquistador produced significantly the largest fruit and Szegedi 20 produced the smallest (Table 1). Although the other yield components were significantly different between cultivars the estimated half product per unit area was not significantly different (Graph 2). Szegedi 20 was the highest in dry matter content and pigment production per unit area (Table 2). Szegedi 80 and Szegedi 20, gave the best pigment content (>10g/kg) which makes them a good source for oleoresin production. Hungarian cultivars in our trials produced better quality than in Hungary, due to the longer duration of sunshine hours and sunlight intensity to which the crop is exposed (Derera et al, 2005).

Conclusions

- Hungarian cultivars were characterised by high commercial yield, synchronised maturity, high dry matter content, and very high extractable pigment content.
- Szegedi 20 and Szegedi 80 produced very high extractable pigment content (>10 g/kg) and the highest pigment production per unit area.
- USA cultivars were characterised by high fresh total yield, strong erect stem, and fruit setting well above the ground.
- The USA and Hungarian cultivars are included in our breading program as parents for their specific traits.

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Acknowledgment

The authors wish to express their gratitude to the Australian government Rural Industries Research and Development Corporation (RIRDC) for financial support and to the University of Sydney, Plant Breeding Institute for providing facilities.

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