Development of New High Yielding Chilli Hybrids (*Capsicum annuum* L.) Based on Heterobeltiosis and Characterization of Parental Germplasm for DNA Polymorphisms

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ABSTRACT. *Twenty-eight hybrids of chilli (Capsicum annuum* L.*) were produced through the half Diallel genetic design using improved chilli varieties, MI 1, MI 2, KA 2, Arunalu, MI Hot, IR, Thiwari and Hot Pepper. Hybrids and parents were evaluated for yield characteristics using a 7 x 7 lattice design under the field conditions of Meewathura farm, University of Peradeniya, from June to November 2004. Performance for total yield, total number of pods, average pod weight, plant height, plant width, pod length, and dry matter percentages were significantly different among tested genotypes. High heterobeltiosis was observed for total yield, while heterosis for total number of pods, average pod weight, and dry matter percentage was low. H 42 was the best performing hybrid with 113.24% heterobeltiosis for total yield having early flowering characteristics. PCR, based on RAPD primers OPM 05 and OPM 11 gave polymorphic banding patterns between the parents which can be used for Quantitative Trait Loci (QTL) analysis in the future.*

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most important cash crops grown in Sri Lanka. It is an essential condiment used in Sri Lankan meals. The estimated per capita consumption of dry chilli is 2.32 kg/annum and around 40,000 mt of dry chilli are required to fulfill the national annual requirement (www.gov.agriculture/agridept.lk). The annual production of dry chilli was 11,700 mt in 2002 and the balance requirement was imported (Central Bank of Sri Lanka, 2002).

The Department of Agriculture has recommended five chilli varieties; MI 1, MI 2, KA 2, Arunalu and MI HOT, none of which are F_1 hybrids. The potential yield of those recommended varieties is 2.5-3.0 t/ha, but the national average yield is 0.75-1.0 t/ha. These low yields are mainly due to low genetic potential of the varieties, high incidence of pest and diseases, moisture stress, use of inferior quality seeds, poor management and high input cost (www.gov.agriculture/agridept.lk).

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The supply of quality seeds of improved varieties to farmers in order to raise the productivity of crops and farmer income has been an issue of national importance (Central Bank of Sri Lanka, 2002). Unavailability of good quality seeds and low productivity of local varieties are some reasons that have moved farmers away from cultivation of local chilli varieties. Farmers tend to grow imported hybrid chilli varieties with high yield potential in productive lands. Since those hybrids are not bred and tested for local conditions, risk of growing such foreign varieties is high. India has overcome a similar problem by producing high yielding local chilli hybrids as an alternative to the existing pure varieties (Hundal and Dhall, 2001). In view of the present local and global situation with regard to production of high yielding hybrids, local production of hybrid vegetable varieties has been identified as a matter of national importance in order to increase the income of farmers.

The possibility of exploiting hybrid vigor and heterosis in chilli hybrids using intervarietal crosses has shown considerable promise (Perera *et al*., 1989). The enormous genetic diversity available for chilli/pepper breeding has facilitated the development of new varieties and hybrids. Several pepper breeders have reported that the level of heterosis exhibited by pepper hybrids is directly related to the genetic distance between parental lines (Nowaczyk and Nowaczyk, 1999; www.apps.fao.org). The search for superior parents in pepper breeding programs is commonly based on the estimation of General Combining Ability (GCA) and Specific Combining Ability (SCA) of inbred parents (Garcia *et al.,* 2002).

Since heterosis of a hybrid is related to the genetic differences between the two parental lines, a wide genetic pool must be exploited for the production of a superior hybrid. To increase the variability among the varieties used for hybridization, unexploited germplasm can be used. In this sense, introduction of foreign varieties to the crossing program is of vital importance. Since less information is available about the performance of crosses of different varieties, the Diallel genetic design is the most appropriate to produce hybrids and to assess heterosis, GCA, SCA and other characteristics of all possible crosses. In Sri Lanka, production of hybrid vegetable seeds is a national priority and therefore, this experiment was conducted to produce superior chilli hybrids using both local and foreign germplasm.

Randomly Amplified Polymorphic DNA (RAPD) markers have been widely used for identifying markers for QTLs and for the estimation of genetic distances among closely related individuals (Garcia *et al.,* 2002). In this experiment, RAPD primers were employed to find polymorphisms between the parents that would be used in future Quantitative Trait Lo (QTL) mapping programs in chilli.

MATERIALS AND METHODS

A total of 28 hybrids were produced using the half Diallel crossing design, employing five locally improved (MI 1, MI 2, KA 2, Arunalu and MI Hot) and three foreign varieties (IR, Thiwari and Hot Pepper) at the Agricultural Biotechnology Center, University of Peradeniya, Peradeniya, in the *Maha* season of 2003. F_1 hybrids and their parents were evaluated for yield and other characteristics using a 7×7 lattice design, including 8 replicates, under the field conditions of Meewathura farm, Peradeniya, from June to

November 2004. Each replicate had two plants each of the eight parents and four selected hybrids and one plant each of the other hybrids and a selected commercial hybrid variety. Each treatment was represented by a single plant and the plot size of each replicate was 5.4 m x 5.4 m. Days to flowering, total yield, total number of pods per plant and dry matter percentage of mature pods were the major characters measured. Pest and disease incidences in the field were regularly inspected and recorded if present. All the agronomic practices were done according to the recommendations of the Department of Agriculture (http://www.gov.lk/agriculture/agridept).

Analysis of variance was performed for each character and least square means were obtained (Johanson and King, 1998). GCA and SCA were estimated through the analysis of Diallel design following the Griffing method, using LS mean values of each character (Mather and Jinks, 1982). All genotypes were ranked according to the mean performance for each character and heterosis was calculated as heterobeltiosis based on the mean value of better parent. Days to flowering were calculated based on 50% flowering.

DNA was extracted using modified CTAB protocol outlined by Samarasinghe *et al.* (2001). Three grams of tender leaves were ground in liquid nitrogen into a fine powder. It was transferred to 15 ml of pre-warmed extraction buffer (4% CTAB, 0.02 M EDTA, 1.4 M NaCl, 0.1 M Tris HCl, 0.1% β mercaptaethanol) and incubated for 30 min. at 60^0C. An equal volume of chloroform:isoamyl alcohol (24:1 v/v) was added and mixed gently for 10 min. Samples were centrifuged for 10 min. at 5000×g. The supernatant was extracted and an equal volume of chloroform:isoamyl alcohol (24:1v/v) was added, after which the samples were centrifuged again for 10 min. at 5000×g. The supernatant was extracted to a 50 ml glass tube and 0.6 (by volume) of ice-cooled isopropanol was added. DNA was hooked out and washed with washing solution (1 M Ammonium acetate: 70% Ethanol 1:99). After drying the pellet at room temperature, 200 µl of TE buffer was added to dissolve the DNA and the solution was stored at 4^0C. The DNA was quantified using UV spectrophotometer and diluted to a final concentration of 20 ng/µl for the PCR reaction.

PCR was performed for the DNA of eight parental varieties using 15 µl of a cocktail mixture (20 ng/µl DNA 4 µl, 10xPCR buffer1.5 µl, 25 mM $MgCl_2$ 1.5 µl, 1.2 µl of 10 mM dNTP mix, 0.2 µl of *Taq* polymerase, 0.8 µl of 10 pmol primer and 5.8 µl of distilled water) with a denaturing cycle at 94^0C for 4 min. followed by 40 repeated cycles at 93^0C for 1 min. at 35^0C for 3 min. and at 72^0C for 2 min. followed by 10 min. of final extension at 72^0C and held at 4^0C (Perera and Jayasinghe, 1998). Amplified products with Techne thermal cycler were separated in a 1% agarose gel, stained with ethidium bromide, visualized under UV light and documented with polaroid camera.

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences among the genotypes for total yield, number of pods, pod weight, and dry matter percentage of pods (Tables 1, 2, 3 and 4). Genotypes were ranked based on the mean performance of each character.

Three hybrids, H 42, H 48 and H 04, showed heterobeltiosis (higher than the mean of better parent MI hot, which was also the highest scoring parent for yield). For yield, H 42

(IR × MI2) was the best hybrid with a mean fresh pod yield of 989.79 g and 113.24% heterobetiosis (Table 1).

H 09 (MI 2 × MI 1) was the best performer for total pod number with a mean of 215.73 (compared to 193.97 of better parent) and 10.8% heterobeltiosis. Among the best hybrids for total pod number, H42 performed well with 179.51 and 22.59% heterobeltiosis and 143.42 SCA (Table 2).

None of the hybrids showed any heterobeltiosis for pod weight. However, H 42 was the only hybrid that showed general heterosis (more than mid-parent value) (Table 3). Among the hybrids, only H 08, H 21 and H 20 showed heterobeltiosis for dry matter percentage (Table 4).

Many hybrids showed heterobeltiosis for earliness to flowering of which H 42, H 17, H 10, H 11 and H 26 were the best five hybrids (Table 5).

As expected and reported by others (Alwis *et al*., 2005; Weerasinghe *et al*., 2004) no single hybrid showed superiority for all the characters (Table 6). In this experiment, H 42 showed heterobeltiosis and was ranked as the best for yield, pod number and earliness to flowering. Therefore, H 42 was identified as the best hybrid among the 28 hybrids evaluated. Based on the mean yield performance, total number of pods and early flowering and early maturing characters, H 42 was the best hybrid out of 28 tested hybrids in this experiment. It was not affected by any pests or diseases in the field. For recommendations to be made, this hybrid will have to be grown in all possible chilli growing areas in order to select the best environment for its cultivation.

Table 1. Mean fresh yield performance and percentage heterobeltiosis of best hybrids.

Hybrid (Parents)	Mean yield of hybrids (g)	Mean yield of better parent	Heterobeltiosis (%)	SCA*
H 42 (IR x MI 2)	998.78	468.38	113.24	615.24
H 48 (MI Hot x KA 2)	819.69	687.76	19.18	155.31
H 04 (Hotpepper x MI 2)	701.06	465.00	50.00	796.96
MI Hot (Best parent)	687.76			

Note: * SCA - Specific Combining Ability

Table 2. Mean pod number and percentage heterobeltiosis of best hybrids.

Hybrid (parents)	Hybrid	Better parent	Heterobeltiosis (%)	SCA
H 09 (MI 2 x MI 1)	215.73	193.97	10.80	55.92
H 25 (MI Hot x MI 2)	191.22	161.05	18.70	146.93
H 42 (IR x MI 2)	179.51	146.06	22.59	143.42

Table 3. Mean fresh pod weight (g) and percentage heterosis of best hybrids.

Hybrid (Parents)	Hybrids	Parents	Heterosis over mid-parent value (%)	SCA
H 15 (IR x MI Hot)	8.96	11.10/8.18	-7.05	-1.55
H 28 (Hot Pepper x MI Hot)	6.18	11.10/4.32	-19.80	4.56
H 39 (Hot Pepper x MI 1)	6.10	11.10/2.49	-10.16	5.20
H 36 (IR x MI Hot)	5.99	8.18/4.32	-4.16	5.20
H 42 (IR x MI 2)	5.86	8.18/3.48	0.51	4.27

Table 4. Mean dry matter percentage of pods and percentage heterobeltiosis of best hybrids.

Hybrid (Parents)	Hybrid	Better parent	Heterobeltiosis (%)	SCA
H 08(Thiwari x Arunalu)	23.76	23.02/20.34	3.21	11.92
H 21 (Arunalu x MI2)	22.25	20.34	9.30	8.35
H 20 (KA2 x MI2)	21.92	19.83	10.53	7.35

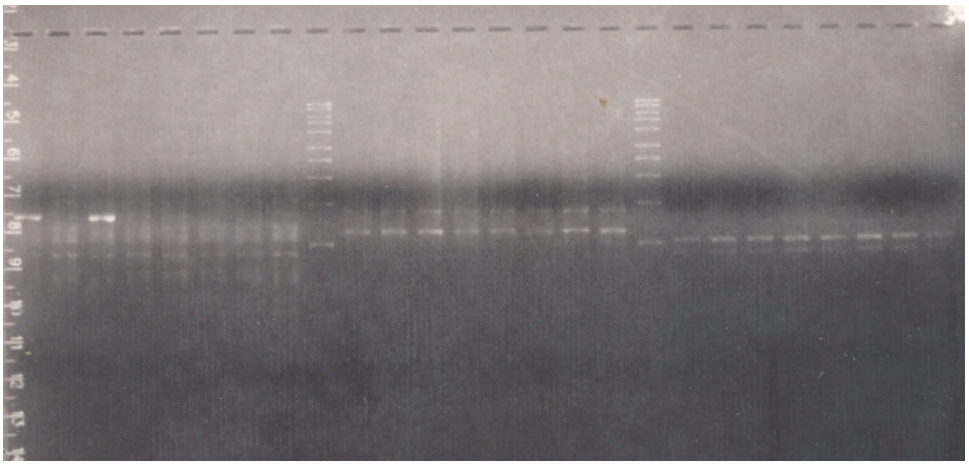
Table 5. Days to 50% flowering of some hybrids and their parents.

Hybrid (Parents)	Days to 50% flowering		
	Hybrid	P1	P2
H 42 (IR x MI 2)	58	64.0	69.0
H 17 (Hot pepper x Arunalu)	58	73.5	64.5
H 10 (IR x Arunalu)	59	64.0	64.5
H 11 (IR x MI 1)	59	64.0	74.5
H 26 (Thiwari x MI 2)	60	75.0	108.5

Table 6. Ranking of hybrids for all characters.

Rank	Yield	Pod number	Dry matter %	Days to flowering
1	H 42	H 09	H 08	H 42
2	H 04	H 25	H 21	H 17
3	H 48	H 42	H 20	H 10, H11

The CTAB method of DNA extraction was successfully applied for the extraction of DNA of chilli and a good yield of DNA was obtained. PCR amplifications were shown by OPM primers 2, 5, 7, 8, 10, 11 and 13. Of these, primers OPM 05, OPM 11 and OPM 13 produced polymorphic banding patterns (Figs 1 and 2). These polymorphic banding patterns of the parents would be used for testing true hybrids and for mapping/tagging QTLs in future experiments.

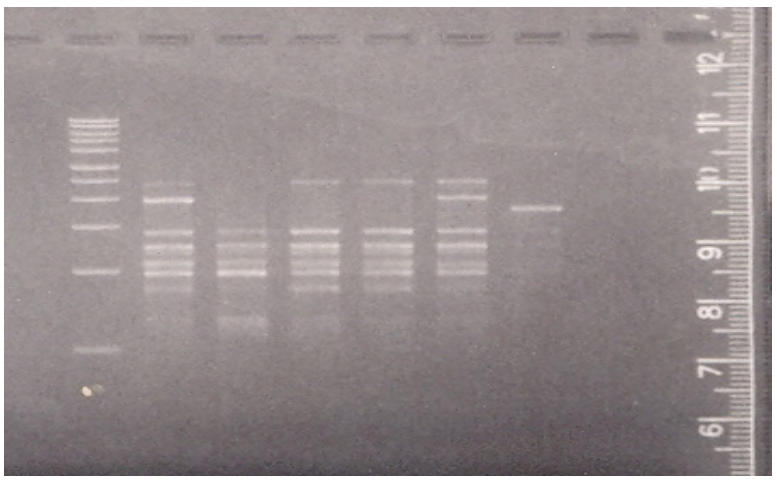


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

Fig. 1. PCR products of chilli varieties.

Note: 1. With primer OPM 05 (lanes1-8), OPM 10 (lanes 10-17) and OPM 08 (lanes 19-26);

2. Lanes 9 and 18 1kb ladder, lanes 1, 10, 19(Thiwari), lanes 2, 11, 20 (Arunalu), lanes 3, 12, 21(KA 2), lanes 4, 13, 22 (MI Hot), lanes 5, 14, 23 (MI1), lanes 6, 15, 24 (IR), lanes 7, 16, 25 (Hot Pepper) and lanes 8, 17, 26 (MI 2)



1 2 3 4 5 6 7 8 9

Fig. 2. PCR products of chilli varieties with OPM 11.

Note: Lane 1, 1 kb ladder, lane 2 (Thiwari), lane 3 (Arunalu), lane 4 (KA 2), lane 5 (MI Hot), lane 6 (MI 1) and lane 7 (IR)

CONCLUSIONS

The hybrid H 42 was selected as the best as it performed better than the parents (currently recommended varieties) in yield, pod characteristics and early flowering. H42 therefore, promises to be a superior hybrid variety, the first of its kind that can be recommended to the chilli farmers of Sri Lanka. In the molecular characterization, the RAPD primers OPM 05, OPM 11and OPM 13 gave reproducible, polymorphic banding patterns among the parents. These would be used to map important QTLs in the future.

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