SABRAO JOURNAL of BREEDING and GENETICS

ISSN 1029-7073; eISSN 2224-8978

VOL. 47 NO. 1 MARCH 2015

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SABRAO
THE SOCIETY FOR THE ADVANCEMENT OF BREEDING RESEARCH IN ASIA AND OCEANIA
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INVITED REVIEW

SABRAO Journal
of Breeding and Genetics
47 (1) 1-13, 2015

RICE BREEDING AT THE CALIFORNIA RICE EXPERIMENT STATION

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SUMMARY

Commercial rice production in California has been underway since 1912 initially relying on selections from japonica plant introductions from Japan and China followed by release of the founding medium grain Calrose in 1948. The establishment of a grower-funded accelerated rice breeding program at the Rice Experiment Station in 1969 has supported California rice production with the release of 45 rice cultivars including medium-, short-, long-grains as well as specialty market types. Support, structure, methods, and objectives of the RES Rice Breeding Program are described.

Keywords: Temperate japonica rice, yield, quality, milling, cold tolerance, disease resistance, grower funded research

Manuscript received: October 1, 2014; Decision on manuscript: October 10, 2014; Manuscript accepted: October 10, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

California rice production

Rice production in California is concentrated in the Sacramento Valley of Northern California. This is a temperate Mediterranean climate with low humidity, essentially no summer rainfall, and relatively high day time temperatures. Low night time temperatures may occur periodically causing pollen sterility and yield loss. Thus indica and tropical varieties have not been successful. Cold tolerant japonica types adapted to a high intensity water-seeded lowland production system on heavy clay soils are grown. Approximately 90% of the California production is planted to medium grains, 8% short grains, and 2% long grains. The production system is highly mechanized which includes aircraft seeding, precision fertilizer application, and chemical pest control. There are relatively few diseases and insect pests, but weed control is a major production challenge and expense for growers. There is organic rice production on about 5% of the area. Planting begins in mid-April and is concluded by early June, with harvest beginning in September and usually completed by early November. The California Rice Industry is subject to considerable government regulations and restrictions; including pesticide registration, use, and application; management practices (burning residue and water management); approval of rice cultivars for commercial production as well as rice research activities (importation of seed into California, transgenic rice and types with potential commercial impact). It is an expensive crop to grow and requires considerable capital

and equipment. California rice growers are typically innovative, quick to adopt new technology, active in the industry and government, and fund the grower-owned Rice Experiment Station (RES) as well rice research by the University of California (UC) and the USDA-ARS.

RICE BREEDING PROGRAM

History

Although rice production for California had been proposed and attempted since the mid-1800s, the event that seems to have launched California rice production and RES was the maturing of a successful rice crop on 16 hectares on the Crane Ranch, southwest of Biggs, California. The 1908 experiment was the work of W.W. Mackie (a soil specialist with the USDA's Bureau of Soils Survey), and led to the founding of RES in 1912. In the previous few years, scientists with the USDA's Bureau of Plant Industries tested and identified a few plant introductions that successfully produced a rice crop in small plantings in the heavy clay soils of the Northern Sacramento Valley. In the following few years, selections of two of the best adapted selections short grain introductions were identified and named 'Caloro' and 'Colusa' (Johnson, 1958). They became the predominant California rice cultivars for the next 45 years.

By the 1930s, there had been rapid development in related disciplines, and applied plant breeding was beginning to emerge and make its mark in areas like hybrid corn and cereal breeding. Genetics, with its laws of inheritance, were better-understood and scientific breeding methods helped develop new cultivars through hybridization and selection. This brought plant breeders knowledge and tools to use "directed evolution" to develop improved cultivars, rather than just relying on introductions from other places and random variability created in materials due to natural out-crossing or mutations. Planned cross-pollination, followed by the natural self-pollination and selection of rice until the traits became fixed, was used to develop rice cultivars in the Southern United States and undertaken at RES. This pedigree breeding method led to the 1948 release of the medium grain 'Calrose' (Johnson, 1958) and continues to be the predominant method for varietal improvement at RES. By 1960, Calrose was grown on 30% of California rice acreage and by 1975 that number had risen to 70%. Although the cultivar Calrose is no longer grown commercially today, the name has become a market class for rice cultivars that have been developed from Calrose with the same or improved medium grain quality characteristics.

Through the 1960s California rice growers began asking for more progress on varietal development. "The Green Revolution" in cereals was starting and California rice growers wanted to participate and remain competitive. There was also a need for expanded rice research to address production issues. Several California grower groups visited the rice research stations in the Arkansas, Louisiana, and Texas and also the newly formed International Rice Research Institute in the Philippines. They recognized the benefits of a well-funded and productive research program and in 1969 established a grower-funded rice marketing order, or check-off. Growers elected to annually collect funds ($0.05/100 kg) based on their production of paddy rice to support research. This provided funds for an accelerated rice breeding program at RES, and breeders began to improve the adaptation and productivity of Calrose as well as develop cultivars for the other market classes (long, short, waxy, etc.).

Organization, funding and facilities

The California Cooperative Rice Research Foundation (CCRRF) is a private, nonprofit research foundation and the membership consists of California rice growers. The Rice Experiment Station is owned and operated by CCRRF. RES was established at its present site between Biggs and Richvale, California in 1912 through the cooperative efforts of the Sacramento Valley Grain Association and the United States Department of Agriculture (USDA). Policy and administration of RES is the responsibility of an 11-member Board of Directors (rice growers) elected by the CCRRF

membership. CCRRF works to serve all California rice growers.

Rice variety development at RES is funded through annual grants to RES by the California Rice Research Board (CRRB) that manages funds received from all California rice producers through assessments on rice production (http://www.carrb.com)**.** The CRRB acts under the authority of the California Department of Food and Agriculture and growers must vote to continue the program every 5 years. The CRRB finances approximately 80% of the total RES annual budget through an annual grant. The remaining 20% is derived from the sales of foundation rice seed to seed growers, grants, and revenues from investments. RES foundation seed production is self-supporting and not funded by the CRRB. RES however does receive grants from the Rice Research Trust (RRT) to fund research capital expenditures and research related activities. The RRT is a tax-exempt trust established in 1962 to receive tax-deductible contributions for support of rice research. RES is not government supported. The RES research budget for 2013 was $2M.

RES facilities are summarized in Table 1. Activities at RES are devoted primarily to breeding and production of foundation seed of RES cultivars for California seed growers. RES has 18 full time employees. The scientific staff includes a Station Director, Director of Plant Breeding, Long Grain Breeder, Short Grain and Premium Quality Breeder, and DNA Lab Research Scientist (PhDs); Rice Pathologist and DNA lab technician (MS); a Breeding Nursery Manager and 4 Breeding Assistants. Operations are supported by a field/maintenance staff of 4 and an administrative assistant. Part-time labor of up to 25 workers is used at planting, harvest, and nursery seed processing. In addition, RES provides research land, facilities, and support to University of California researchers in weed science, agronomy and entomology at no charge.

Breeding methods

The RES plant breeding program aims to develop rice cultivars of all grain types and market classes with high and stable grain yields and quality that will sustain the profitability of rice with minimum adverse environmental impact. Important breeding objectives include the incorporation of high yield potential, seedling vigor, cold tolerance, early maturity, lodging resistance, and disease resistance into future rice cultivars. Improved milling yield, grain appearance, and cooking characteristics relative to consumer preference, are also major components of the plant breeding program.

Table 1. Land and facilities of the Rice Experiment Station.

Land	Area (ha)	Comment	Facilities	Area (m^2)
Total Facility	193		Greenhouses	
			5 Breeding& Pathology	1100
			1 Weed research	210
Breeding Nursery	30	1 year rotation	2 Cold Screening Greenhouses	115
Other Nurseries	18	Weed, Agronomy, Insects research	Research Building and Seed House	1200
Foundation Seed	83	Some fields fallowed annually	DNA, Pathology, Quality, Weed labs	210
Cold Tolerance Nursery	2	Grower field	Seed Drying, Storage and Cleaning	850
Hawaii Winter Nursery	1	1 year rotation	Office, Shop and Storage	1615
			Seed Drying/Storage Bins (18-127 Mg and 5-25 Mg)	

The breeding program has basically followed a pedigree method, pure line selection with some modifications, and some backcrossing. Induced mutation, especially for semi dwarfism and some endosperm traits has been used directly and through cross breeding. Crosses are made in greenhouse independently in spring and fall by each project. Crossing blocks are planted over about 4 dates comprising of ~100 potential parents. Plant breeders make daily selections of the crosses to be made at flowering. Each new cross is assigned a unique continuing consecutive R# after the season is complete and seed is set and harvested. F_1s are then grown by transplanting in the field in the summer or Hawaii nursery, or may go back to the greenhouse for crossing. F_2 populations are grown at RES and may be duplicated at cold tolerance nursery (San Joaquin County). Greenhouses are used for rapid generation advance and cold tolerance screening. Grain and quality types/objectives within projects are grouped together throughout the rest of the breeding and nursery process. Bundles of panicles for advancement are selected from the F_2 populations for lab examination as brown rice during the winter. From these F_2 panicles, a panicle-to-row advancement process proceeds involving several panicles being selected from a row and advanced or discarded by visual or other criteria. The program has developed the capability to grow a large water-seeded nursery. This allows selections and evaluations in the commercial production system used in California from the F_3 generation forward. After a few generations, the row may be cut (after panicles are selected) for quality testing and the seed from the row used to begin small plot testing. Small plots are assigned to each project, and may or may not be replicated and have many checks included. Preliminary yield tests are 4 replicates planted at 2 seeding dates at RES (3 x 6 m plots) and a drilled seed maintenance plot is grown for a pure seed source. Statewide Yield Test entries are grown in the same manner and are conducted by the University of California Cooperative Extension. Table 2 presents a summary of activities in the breeding program and Table 3 contains an example report summary of one of the maturity groups from the 2012 UC Statewide Yield Tests.

Yield

The climate, soils, and production system of California's rice growing regions do support high field yields with state rice yields averaging over 9 t ha^{-1}. Since the establishment of the RES accelerated breeding program in 1969, statewide average yields have increased from 6.1 to 9.3 t ha^{-1}. Yield potential continues to be a primary breeding objective at the RES. Yield gain in US rice was recently reviewed (McKenzie *et al*., 2014). H.L. Carnahan (RES Director of Plant Breeding 1969-89) estimated that 60% of this increase was attributed to improved rice cultivars (McKenzie *et al*., 1994). Genetic gain was estimated at 25 and 50 kg ha^{-1} yr^{-1} for the periods 1981-2011 and 1996-2011, respectively (McKenzie *et al*., 2014).

With the relatively high yield potential, achieving incremental yield increases are becoming more difficult and there is growing concern about reaching a yield plateau. Experimental materials however are still being recovered with increased yield potential. Hybrid rice, as an approach to increase yield, has been considered, but RES has not initiated a breeding effort in this area. Obstacles to hybrid rice development for RES include: (1) a temperate japonica cultivar base with low heterosis, (2) already high commercial yields with current inbred cultivars, (3) cold tolerance, (4) limited acreage and resources to support a hybrid breeding effort, (5) a water-seeded production system requiring high seeding rates, (6) grain quality and market acceptance, and (7) seed production and seed import restrictions (McKenzie *et al*., 2014). Non-transgenic herbicide tolerant rice (Clearfield ™) has been widely adopted in the southern US and contributed to increased commercial yields in that region. While weed control is a major issue for California rice growers; this technology has not been made available in California. Improvements using transgenic technology would currently be rejected as unacceptable by marketers and thus California growers. Marker-assisted selection is being used at RES, but currently only for grain quality factors and for blast resistance that involve major genes, but it is hoped that it may contribute to facilitating further yield increases in the future.

Table 2. Summary of RES 2012 rice breeding and seed production activities.

2012 Nursery	Number	Method	Plots	Location	Comments
Crosses	1418	Vacuum-approach	4 Crossing blocks in	Greenhouse	Primarily 3 way, BC, and
F_1	1400	Transplant	>5 space planted	RES/Hawaii	Single to BC/3 way
F_2	RES-1685	Precision	12 x 1.8 m	RES/Cold	Brown rice visual selection
	Cold Nursery-960	Drilled	0.15 m spacing	nursery	
F_{3-5}	~70,000	Water seed	1.2 m row	RES/ Hawaii	Brown rice visual selection
		vials	0.5 m spacing		
Small plots	1915	Water seed	1.2 x 1.8 m	RES	Brown/milled/cooking
	2112	Vials/sacks	3 x 3 m		
Preliminary yield test	959 entries	Water seed	3 x 6 m	RES	Brown/milled/cook/
		sacks	2 reps		Harv. H_20 /cold
Statewide	44 Adv. entries	Water seed, sacks	3 x 6 m	UC/Growers	Brown/milled/cook/Harv.
	96 Pre. entries	1 drilled	Adv. 4 rep	& RES (7)	H_20/cold tolerance
			Pre. 2 rep		
Experimental	5		1.5 m row	RES	Brown/milled/Harv. H_20
Headrow		vials	0.5 m spacing,		/cold tolerance/cook/ market-
Breeder		sacks	Broadcast		quality
Foundation Seed	15 cultivars	Dry seed to	68 ha	RES	Seed allocated to CA
Production (68 ha)		permanent flood			growers by CA Crop
	4 headrows	Water seeded rows	400 per variety		Release & IP Protection
	2 experimentals	Water seeded rows	Breeder increase		

Table 3. Very early advanced variety tests (4 location average) in UC cooperative extension statewide yield tests (2012).

Entry	Grain Type[1]	Grain Yield (kg/ha)	Harv. H_20 (%)	Seedling Vigor[2] (score)	Days to 50% Heading	Lodging[3] (%)	Height (cm)
09Y2141	SWX	11220	20.9	4.9	90	35	97
M206	M	10820	20.6	5.0	92	20	94
09Y2036	S	10760	19.1	4.9	89	60	97
10Y3286	M	10750	19.0	4.9	88	2	91
11Y1005	L	10620	18.0	4.8	93	2	94
08Y3310	M	10520	20.8	5.0	94	1	89
M104	M	10450	19.6	5.0	86	10	91
08Y2049	SSR	10350	19.5	4.1	89	10	86
06Y575	L	10320	19.0	4.0	99	10	97
CH202	SWX	10160	17.9	4.9	89	60	86
L206	L	10140	16.8	4.6	92	1	81
08Y3269	M	10140	21.7	4.9	96	25	94
M202	M	9880	21.4	5.0	95	20	97
CH201	SPQ	9770	17.6	5.0	94	50	89
S102	S	9640	15.6	5.0	84	30	91
M205	M	9250	22.8	5.0	99	25	89
CM101	SWX	8770	16.5	5.0	85	35	91
MEAN		10200	19.2	4.8	91	23	91
CV		4.7	5.5	2.5	1.2	66.4	4
LSD (.05)		340	0.7	0.1	0.1	NA	2.5

[1] M = medium grain. S = short grain, L = long grain, SWX = short grain waxy, SPQ = short grain premium quality, SSR = Stem rot resistant
[2] Subjective rating of 1-5 where 1 = poor and 5 = excellent seedling emergence
[3]Subjective rating of 1-99 where 1 = none and 99 = completely lodged.

Cold tolerance

Seedling vigor, facilitating rapid emergence for the water seed production system, and resistance to low temperature induced pollen sterility prior to heading are important cold tolerance traits needed in California rice cultivars. Visual evaluation on water-seeded rows or plots and seedling vigor scores (Table 3) are collected on the breeding materials from the F_3 generation forward. The shift to semi dwarf cultivars posed a concern that these types would have considerably weaker seedling vigor. Some initial studies (McKenzie *et al*., 1980) suggested this would not be a problem in RES germplasm and this has proven to be the case in the medium grain project. When foreign germplasm has been introduced as a parent, particularly in the short grain and long grain projects, low levels of seedling vigor have been encountered. Most RES germplasm has acceptable level of seedling vigor for water seeding. Efforts were made to achieve high level of seedling vigor observed in introductions, like Italica Livorno (Williams and Peterson, 1973). This was not successful after several attempts at RES as the material

recovered was very early maturing, lodged severely, and stem rot susceptible making it unacceptable for release. The wide spread use of precision laser leveled fields by growers (providing improved water depth control), has lessened the demand for higher level of seeding vigor in new cultivars. The breeding program also has begun monitoring advanced lines for phytotoxicity to several of the rice herbicides that are applied at planting or in the first two weeks after water seeding.

Resistance to low temperature pollen sterility (blanking) has been a necessity in California rice due to low nighttime temperatures (< 13° C) that can periodically occur at about two weeks before heading. F_2 populations are grown at a cold nursery location and low blanking lines selected. Refrigerated greenhouses at RES are used to evaluate and select advanced lines for resistance to blanking. The Hawaii winter nursery is also a low temperature location used for selecting and evaluating blanking tolerance. Cold tolerance is a difficult trait to evaluate, but through selection in these nurseries over the years quantitative improvements have been made in all grain types. 'Calmochi-101' (Carnahan *et al*., 1986) is still considered one of the most blanking resistant cultivars, and some of this tolerance is credited to a parent 'Tatsumimochi' from Hokkaido. 'M-104' (Johnson, 2002), 'M-206' (Johnson, 2005) and 'S-102' (McKenzie *et al*. 1997) show less blanking than their predecessors although it is difficult to quantify. The release and commercial production of long grain cultivars in California, like 'L-206' (Jodari, 2008), reflects the improvements in cold tolerance that has been made in long grains. Specialty grain types, like aromatics, still represent a continuing challenge for blanking resistance and are restricted to the warmer production areas of California. Efforts have been made recently to evaluate a few elite materials from the Cold Tolerance Group of the Temperate Rice Research Consortium (TRRC) at RES. High levels of blanking were observed in these materials at the Hawaii winter nursery, RES cold tolerance nursery, and refrigerated greenhouses. Likewise, evaluations of some of RES most cold tolerant materials by members of the TRRC group demonstrated little blanking resistance in their screening nurseries. This illustrates the complexity and difficulties often encountered in breeding for cold tolerance.

Quality

Breeding for rice quality in the USA was previously reviewed (McKenzie, 1992). Table 4 contains a summary of grain quality evaluations currently used by the RES Rice Breeding Program. Beginning in the F_2 generation, harvested panicles are discarded that show high blanking or poor panicle characteristic and those selected are hulled and examined as brown rice. This allows the breeder to evaluate the size, shape, defects, and translucency (chalk) of the kernels. It was shown that there was a very high correlation ($R^2 > 0.9$) between the number of broken brown rice kernels and the whole kernel white rice milling yield (McKenzie, 1992b). Actual milling tests (% whole kernel and % total milled rice) are conducted in progeny rows (F_4 or higher). Milling tests are conducted on advanced lines over a series of harvest moistures. The harvest moisture milling studies identify lines with high and stable whole kernel milling yields. Figure 1 shows the results of harvest moisture studies on an experimental line 05Y471 compared to its medium grain parent cultivars M-104 and M-206. 05Y471 which was later released as 'M-105' (Johnson and McKenzie, 2013), shows a much higher and more stable whole-kernel milling yield than M-104. A progression of improvements in the milling yield of the long-grain releases has been achieved as well.

Physiochemical tests like apparent amylose content and alkali spreading value (gelatinization temperature type) are determined in all grain types in advanced generations. There is limited variation in these traits and also in cooking quality performance in the standard medium and short grains. Thus, testing for these traits is only done for verification or when exotic germplasm is used. Physiochemical tests including starch viscosity (RVAs) and cooking tests do receive more emphasis in the long grain and specialty types. DNA markers are used in the long grain project for apparent amylose content class, gelatinization temperature type, and RVA profile. Micro-cooking taste tests (12 g) followed by cooking and testing advancing

Table 4. Quality evaluation testing by grain type in the RES rice breeding program.*****

Grain Type	Size, Shape and Appearance	Milling Yield	Amylose, ASV., RVA viscosity	Cook Test	Other	Marketer & Expert Evaluation
Medium	x	x	x	x		x
Short	x	x	x	x		x
Premium	x	x	x	x x x	protein	x x
Long	x	x	x x	x x x		x
Aromatics	x	x	x x	x x x	2-AP, elongation	x x
Others	x	x	x	x		x x

*Lab testing for amylose and gelatinization temperature type (ASV) provided by USDA Rice Quality Unit; protein from the California Wheat Commission Milling and Baking Lab; RVA, Cooking tests at RES; 2-AP and aroma testing USDA, UC Davis, and RES.

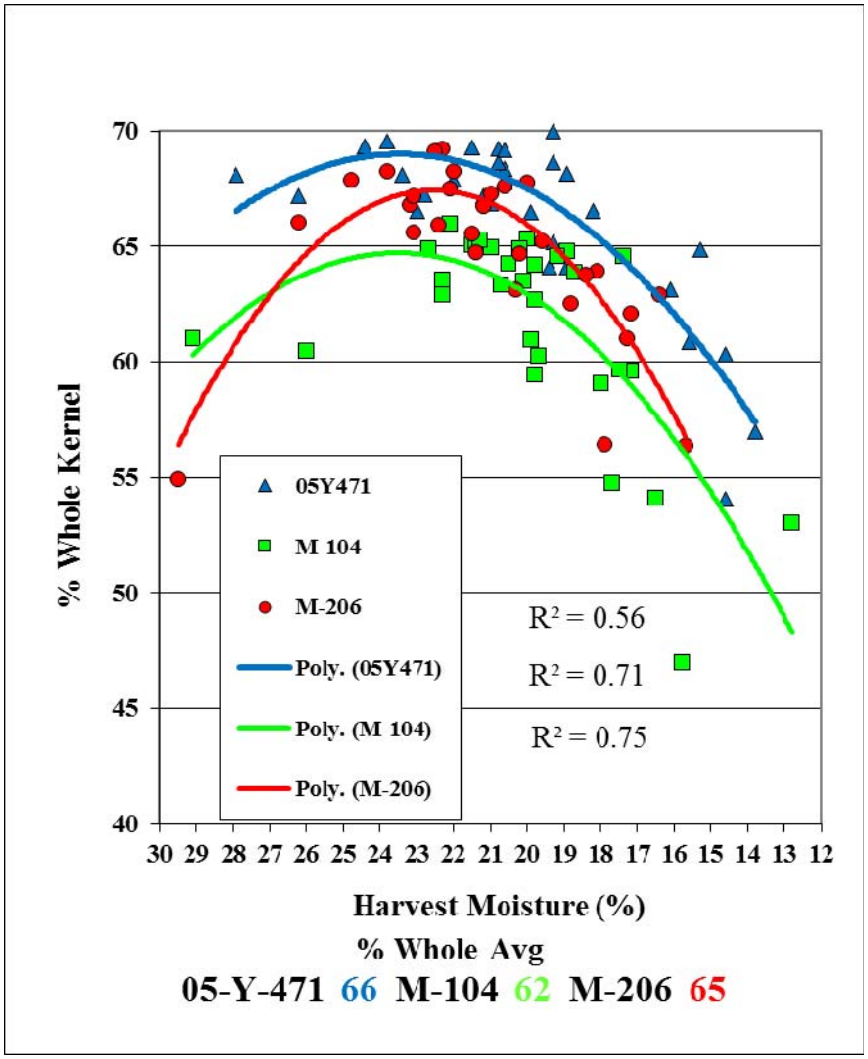

Figure 1. Harvest moisture and milling studies of medium grains 05Y471 (M-105), and its parents M-104 and M-206 at RES in 2009-2010.

lines in electric rice cookers (150 g), are done in house by the breeders. Samples are distributed to marketing organizations or outside experts when a line is under consideration for seed increase and possible future release.

Disease and insect resistance

In 1980, a rice pathologist was hired to support the breeding program and address problems with fungal diseases, particularly stem rot (*Sclerotium oryzae*). No effective fungicides were registered or available at that time. New semi dwarf cultivars were becoming popular and burning of rice straw for disease control and straw management was on its way out. Stem rot resistance, unfortunately, was only identified in an accession of the wild rice species *O. rufipogon* (Figoni *et al*, 1983). Screening efforts were developed (Oster, J.J. 1990). However, transfer of this resistance into adapted material has been a slow and arduous process. The first milestone was the recovery of the resistant long grain line 87Y550 (Tseng and Oster, 1994). Some tolerant short grain lines were recovered from crosses to the high yielding long grain lines derived from 87Y550 in the late 1990's, and these were used in the medium grain project. Efforts to recover this tolerance in to adapted medium grains have only recently been achieved. Materials recovered in the 3-grain types to date, do show improved resistance to stem rot and aggregate sheath spot *Rhizoctonia oryzae-sativae.* Unfortunately, lines suitable for release as cultivars have not been recovered due to weakness (i.e. low seedling vigor, grain quality weakness, and or blanking susceptibility). Studies are continuing on identifying markers for stem rot resistance that can be used for marker-aided selection to achieve this breeding objective.

Blast, *Pyricularia grisea* [race IG-1] was first found in California in 1996. California cultivars are very susceptible to blast and are used as susceptible spreaders in the rice breeding program in the southern US where blast is a major disease issue. Fortunately, the low humidity climate of California's rice growing regions is "permissive but not conducive" to this disease. M-208, released in 2005 contains a major blast resistance gene *Piz*, but recently has been observed to have blast at a low incidence in some fields, determined to be a second race IB-1. A backcrossing project was initiated by the RES pathologist in 2005, to incorporate the different blast resistance genes (Pi genes) into M-206 background. Breeding lines were selected and advanced using DNA markers linked to these genes, and the end-result were 10 near-isogenic lines (NILs) of M-206 containing individual Pi genes. Some of these lines are in the yield testing stage and are being used to pyramid genes into adapted germplasm.

The major insect pest of rice in California is the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel). Root pruning by feeding larvae may be severe, resulting in stunted plants and decreased grain yield and quality. This pest has been controlled with registered insecticides. The breeding program began efforts to transfer tolerance to this insect pest from PI 162254 (WC 1403) identified by entomologists. Screening was done in a field that annually experienced high water weevil pressure with selection for lines with less yellowing and reduction in vegetative height. PI 506230 was released in 1987 as a water weevil tolerant germplasm (Tseng *et al*., 1987) and breeding efforts continued into the early 2000s. Materials continue to show improvement but could not match the yield and agronomic performance of the improved medium grains. With effective commercial insecticide availability, application and damage limited to the field margins, variable infestations, and the identification of other breeding priorities, the efforts in this area were discontinued.

Cultivar releases and pedigrees

Proposal for seed increase of a line is made by the breeder in his annual oral report to the Board of Directors in January. The merits, performance data for review, purity and uniformity, and market evaluation of the line is presented and with the Board approval, putative foundation seed is grown that is inspected, harvested and cleaned that season. The following year, the breeder will submit another proposal and data to the Board for review, approval and release. A cultivar name is proposed and the Board votes to release and determines intellectual property

protection level (PVP and/or Utility Patent). The designated naming system uses the first letter to indicate grain type (M = medium, S = short, L = long, or specialty type e.g. A = aromatic); maturity group 100 s to 400 s for very early to late, respectively; and 1-99 the release number. All cultivars in commercial production in California are reviewed under California state law to determine commercial impact of any trait they might have (aroma, colored bran, or genetic engineered traits [none in the US]) and if production restrictions are required. A review by a California Crop Improvement Association (CCIA) technical committee approves the cultivar for certified seed production. Allocation of foundation seed is made to California rice seed growers by CCIA. The seed is sold in bulk from RES and used to produce registered seed that can be used to produce certified seed the following year. RES breeders are responsible for headrow planting, purity and storage of seeds for breeder seed production in their project's grain type. Cold storage is used to save seed, allowing headrows used for breeder seed planting to be done every 3 years.

Forty-five new rice cultivars have been released by RES to California rice growers since the accelerated breeding program was initiated in 1970 (Table 5). These have included traditional California medium (Calrose market type), short and long grain, as well as specialty types including waxy, aromatics, and premium medium and short grains. RES rice cultivars constitute 90% of California's rice production. Reviews of US rice cultivars have been made by Mackill and McKenzie (2002) and Moldenhauer *et al.* (2004). Figure 2 shows the ancestry of RES cultivars. Selections from introductions from Japan and China were the founding cultivars. Breeding material from the southern US were used in crossing, followed by 'IR- 8' and induced mutations at the start of accelerated breeding effort. Long grains were developed later using long-grain breeding material from the southern US and plant introductions. The germplasm base is quite narrow (Dilday, 1990). Widening the germplasm base is difficult due to strict market and grain quality requirements, as well as adaption to the California rice production system and environment.

Table 5. Cultivars releases since the initiation and funding of an accelerated breeding program**.**

Name	Type	Year	Name	Type	Year	Name	Type	Year	Name	Type	Year
CS-M3	M	1970	S-201	S	1980	S-101	S	1988	M-402	P	1999
CS-S4	M	1972	M-302	M	1981	M-103	M	1989	M-104	M	2000
S6	S	1975	M-401	P	1981	S-301	S	1990	M-205	M	2000
M5	M	1975	Calmochi-202	W	1981	L-203	L	1991	M-206	M	2003
Calrose-76	M	1977	M-201	M	1982	M-204	M	1994	M-207	M	2005
M7	M	1978	L-202	L	1984	S-102	S	1996	M-208	M	2006
M9	M	1978	Calmochi-101	W	1985	A-201	A	1996	L-206	L	2006
L-201	L	1979	M-202	M	1985	L-204	L	1996	Calmati-202	A	2006
Calmochi-201	W	1979	A-301	A	1987	L-205	L	1999	Calamylow-201	W	2006
M-101	M	1979	M-102	M	1987	Calmati-201	A	1999	M-105	M	2011
M-301	M	1980	M-203	P	1988	Calhikari-201	P	1999	Calhikari-202	P	2012
									A-202	A	2014

*M=medium grain, S=short grain, L=long grain,
A=aromatic, W=waxy, and P=premium quality.

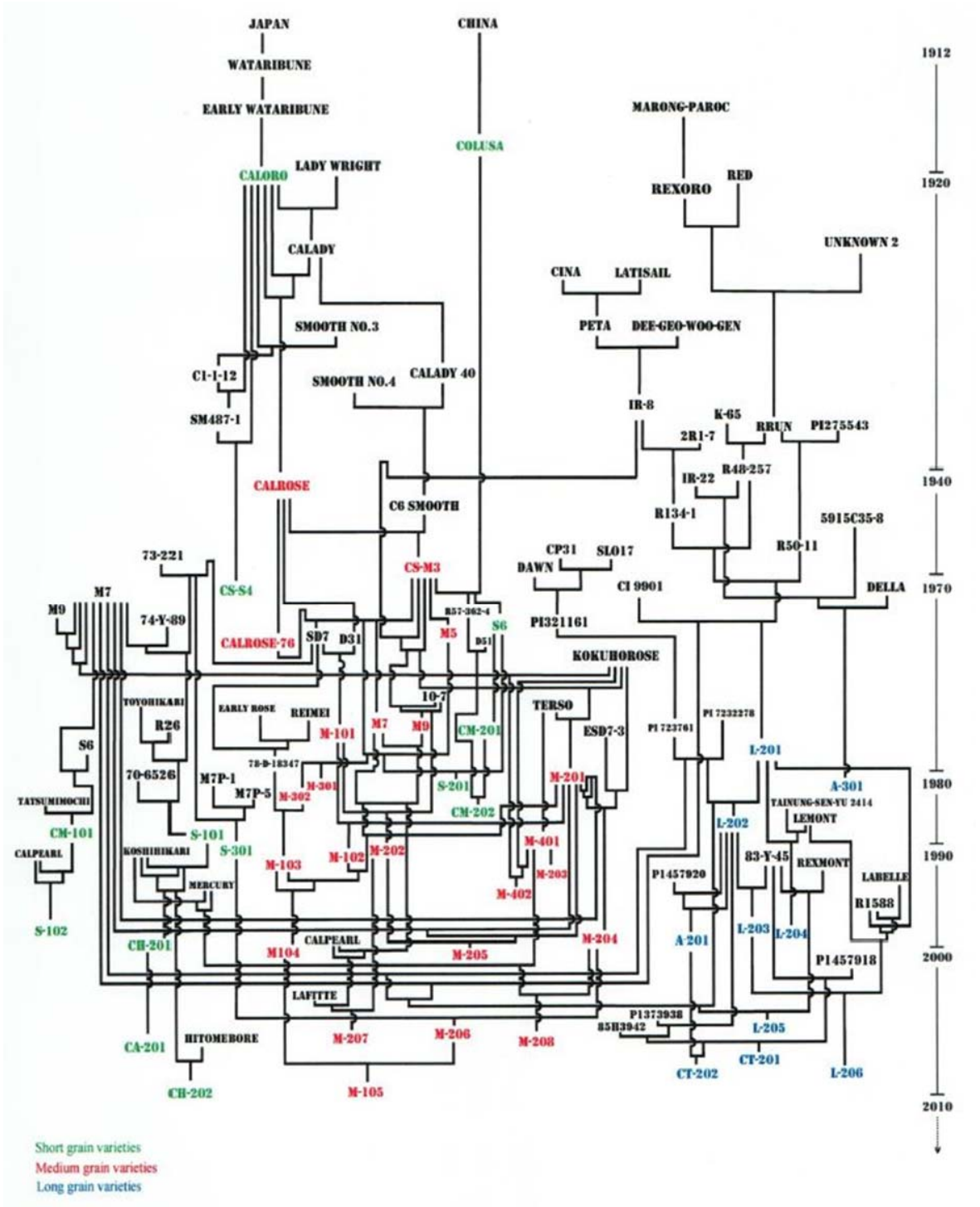

Figure 2. Pedigrees of RES cultivars.

Future outlook and challenges

Future prospect for US rice yield increases were recently discussed by McKenzie *et al.* (2014). Currently the RES breeding projects are producing lines that show incremental improvements in yield over existing cultivars. Capturing the agronomic and quality requirements for California rice in higher yielding lines is a formidable challenge. Considerable effort is being made in evaluating milling yield and grain quality in the selection process in all projects to address quality requirement for new cultivars. Culinary characteristics (aroma, texture, and taste) are critical evaluations that are especially important for the aromatic types and premium quality short and medium grain. Screening and selecting material in cooking tests are resource and time demanding, rely on subjective evaluations, and complicated by environmental effects including post-harvest processing. The pyramiding of resistance genes for rice blast is well underway in medium grains and can be expected to produce adapted cultivars with multiple blast resistance genes. Efforts at RES are being made in the hopes of developing non-transgenic herbicide tolerant mutants for weed control. The challenges in this area include selection of suitable herbicide, recovery of and acceptable mutant(s), weed control and production system verification, weed resistance stewardship program, herbicide registration and approval, and a commercial sponsor. In California, water is a premium commodity, and as in the case of the current drought, the planting acreage rice is reduced to ensure an adequate supply to produce the crop. UC research on water use at RES and the prospects for improving water use efficiency is not encouraging for commercially viable rice production in California. All these activities will need to be done under the prospect of climate change. Selecting in the nurseries environmental conditions for performance and adaptation are standard practice in the breeding process. And finally the RES Rice Breeding Program will need the continued support and funding of growers, the UC and USDA-ARS research, and an innovative and productive staff to continue forward in its 2nd century.

Additional information about the RES Rice Breeding Program including annual reports, field days, a centennial program, and other information are available online at http://www.crrf.org/. Information on California rice production from the University of California is available at the following website: http://ucanr.edu/sites/UCRiceProject/.

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SHORT COMMUNICATION

SABRAO Journal
of Breeding and Genetics
47 (1) 14-20, 2015

GENETIC DIVERSITY OF *Brassica napus* USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

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SUMMARY

Genetic diversity was studied in 7 genotypes of *Brassica napus* for total seed protein content using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Ten reproducible bands were used for cluster analysis and to estimate the genetic diversity. Out of these bands, five bands were observed polymorphic. A dendrogram was constructed and the genotypes were divided into two main groups comprising 4 clusters. The results obtained from these clusters showed genetic diversity in these accessions on SDS-PAGE level. The results showed that the technique of SDS-PAGE was feasible to distinguish the different species of *Brassica napus* genotypes.

Keywords: *Brassica napus,* sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), genetic diversity, cluster analysis

Short summary statement: Characterization on the basis of proteins and selection of desirable lines/genotypes is great importance for rapeseed breeders. The highest similarity was recorded in RSPN-25 and RSPN-29, followed by RSPN-28, GSL-1 and DGS-1, GSL-1 (89%). However, the lowest similarity estimates (63%) were recorded in GSC-101 and HNS-0901, followed by 67% in GSL-1 and HNS-0901. The diverse accessions may be used for crossing in breeding programs.

Manuscript received: May 13, 2014; Decision on manuscript: October 28, 2014; Manuscript accepted: January 8, 2015.

Communicating Editor: Bertrand Collard

INTRODUCTION

Brassica is an economically important genus in the *Brassicaceae* family (Syn. Cruciferae). *Brassica* species are widely used in the cuisine of many cultures as recognized as a valuable source of dietary fibre. *Brassica* vegetables contain little fat and are sources of vitamins, minerals and fiber. Oleiferous brassica species viz. *Brassica napus, Brassica campestris, and Brassica juncea* constitute the world's third most important source of edible oils (Gupta and Pratap, 2007). Among the oilseeds in India, *Brassica* ranks second in area and production after groundnut and contributes 26% after total vegetable oil seed output. Among the 3 cultivated species, *Brassica napus* (AACC 2n = 38) is the most important oileferous crop and it is gaining importance because of its high yield potential of 20 qha^{-1} (Anonymous, 1991), wide adoptability and high oil content (44.6%) and good quality (Rai *et al.,* 2007). Rapeseed oil

has a high concentration of oleic acid (60%), and contains moderate levels of linoleic acid (20%) and linolenic acid (10%). This fatty acid composition of a vegetable oil is considered by many nutritionists ideal for human nutrition and superior to that of many other plant oil (Rakow and Raney, 2003). Rapeseed oil also has the lowest saturated fatty acid content of any vegetable oil, amounting to about 7% of total fatty acids, whereby palmitic acid with about 4% and stearic acid with about 2% of the total fatty acids, are the major saturated fatty acids in rapeseed oil (Adamska *et al*., 2004).

In India, rapeseed mustard is cultivated in an area of 6.51 million ha with a production of 7.67 million tons and with an average yield of 1179 kg/ha. In Jammu and Kashmir (India) 60,000 ha area cultivated under rapeseed-mustard with a production of 48,000 q and 788 kg/ha productivity. (Anonymous, 2010). Looking at the production and productivity of Indian mustard, there is an urgent need for the improvement of *Brassica* crops. In general, genetic improvement of crops can be accelerated when broad genetic diversity and the information of these genetic resources are available. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varietal improvement. Quantitative genetic markers are helpful in the estimation of genetic variation. It is helpful in appropriate use of gene pool in specific programs (Sadia *et al.,* 2009). Characterization on the basis of proteins and selection of desirable lines/genotypes is great importance for rapeseed breeders. The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.,* 2001; Turi *et al.,* 2010). Seed protein is not sensitive to environmental fluctuations and banding patterns therefore are very stable, and can be used for cultivar identification (Tanksley and Jones, 1981; Nasr *et al.,* 2006; Rai *et al.,* 2011). However, the information on the use of SDS-PAGE on different species of *Brassica* for genetic diversity is still limited (Rahman and Hirata, 2004). Therefore, this study was undertaken to assess the protein polymorphisms and determine the genetic diversity of *Brassica* species using SDS-PAGE.

MATERIALS AND METHODS

Plant Material

Brassica napus (seeds) were collected from Division of Plant Breeding and genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India. Seven genotypes/varieties of *Brassica napus* were electrophoretically characterized using SDS-PAGE at the School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. Genotype/varieties are shown in Table 1).

Table 1. List of *Brassica napus* genotypes used.

Genotype	Source
DGS-1	SKUAST-Jammu
GSL-1,	SKUAST-Jammu
RSPN-25	SKUAST-Jammu
RSPN-28	SKUAST-Jammu
RSPN-27	SKUAST-Jammu
HNS-0901	SKUAST-Jammu
GSL-101	SKUAST-Jammu

Protein extraction and purification

Collected seeds of 7 genotype viz., DGS-1, GSL-1, RSPN-25, RSPN-28, RSPN-27, HNS-0901 and GSL-101 were crushed and grounded with the help of mortar and pestle using CTAB method (Doyle and Doyle, 1987). The seed flour was taken in to a 10 ml test tube. A volume of 5 ml of chloroform, methanol and acetone mixture (2:1:1) was added and mixed well by vortexing. Then the samples were kept at room temperature for overnight. After centrifuging the sample the solvent was removed and taken the defatted seed powder was placed in 1.5 ml eppendorf tubes.

Then the protein extraction buffer (0.6M Tris HCL buffer-pH 6.8 mixed SDS and β-mercaptoethanol) was added. Bromophenol blue was added to extraction buffer as a dye to point out the movement of protein in the gel. All these chemicals were mixed together then the solution was purified and homogenated. The samples were thoroughly by vortexed and centrifuged at 12,000 rpm for 10 minutes at room temperature (RT). After centrifuging the samples, the crude protein recovered as clear supernatant on the top of the tube. Then supernatant were transferred into new 1.5 ml eppendorf tubes and stored at -20^0 C until gel electrophoresis. Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970).

Electrophoresis

Crude protein samples were directly analyzed by SDS-polyacrylamide gel electrophoresis using 12.0% polyacrylamide as resolving gel and 4.5% stacking gel. 20 µg protein samples were loaded with the help of micropipette into the wells of the stacking gel. Electrophoresis was carried out at 20 V for staking gel and 100 V for as resolving gel, until the bromophenol blue (BPB) reached to the bottom of gel plate.

Staining and Distaining

After completion of electrophoresis, the gels were placed in fixing solution (15% TCA) in staining box for overnight. After decanting the fixing solution, pored the 2.0% (w/v) coomassie brilliant blue (CBB) R250 in box. When the staining procedure was completed, then the gel was de-stained by washing with a solution containing acetic acid, methanol and water in the ratio of 5:20:75 (v/v), so that the blue color of the coomassie brilliant blue (CBB) R disappears and the electrophoresis band on gels clearly visible.

Scoring of data and analysis

The protein bands were scored as 0 for absence or 1 for presence for polymorphism. The Jaccard's similarity index was calculated using NTSYS-pc version 2.02e (Applied Bio-Statistics, Inc., Setauket, NY, USA) package to compute pair wise Jaccard's similarity coefficients and this similarity matrix was used in cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) and sequential, agglomerative, hierarchical and nested (SAHN) clustering algorithm to obtain a dendrogram.

RESULTS AND DISCUSSION

Bulk seed samples of 7 *Brassica napus* genotypes were used for total protein comparisons. The banding pattern of some of the total seed protein showed close relationship among these studied genotypes, while the difference in banding pattern showed the range of geographic differences (Figure 1 and Table 1). Total 10 bands were detected and which in 5 bands were observed in all genotypes. The ratio of the polymorphic banding was 50%. However, dendrogram was constructed on the basis of these polymorphic bands.

After the study of the banding patterns in these genotypes, 3 zones were observed (A to C) showing variations. As a result, Zone-A was having protein weight from 225 - 90 kDa. Zone-A having total 4 bands which in 2 bands were polymorphic. However, protein bands in Zone-B ranged from 75 to 25 kDa. Protein bands detected in this region were 4, among which 50 kDA protein band were polymorphic. Zone- B comprised both light and dark stained bands, while Zone-C ranged from 15 to 10 kDa. Two Protein bands were produced in this region and single protein band (15 kDA) showed polymorphic (Table 2).

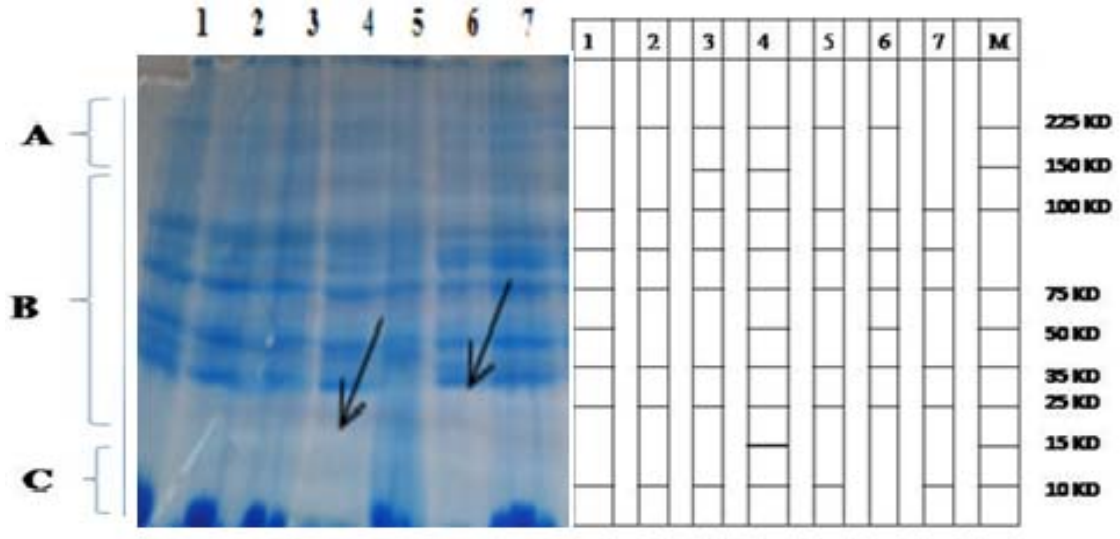

Figure 1. Zymogram of various cultivars of *Brassicas napus* based on SDS-PAGE of total seed proteins. Lane 1 RSPN-28, Lane 2 RSPN-29, Lane 3 DGS-1, Lane 4 GSL-1, Lane 5 RSPN-25, Lane 6 HNS-0901, Lane 7 GSC-101respectively, and M-Molecular weight marker.

Table 2. Presence and absence of protein bands in SDS-PAGE analysis of *Brassica napus.*

Protein region	Protein bands	No. of genotypes/varieties	
		Presence	Absence
GROUP-A	1	6	1
	2	2	5
	3	7	0
	4	7	0
	5	7	0
GROUP-B	6	3	4
	7	7	0
	8	7	0
GROUP-C	9	1	6
	10	6	1

Table 3. Dice Coefficients between different protein types based on SDS-PAGE.

	RSPN-28	RSPN-29	DGS-1	GSL-1	RSPN-25	HNS-0901	GSC-101
RSPN-28	1.00						
RSPN-29	0.88	1.00					
DGS-1	0.78	0.88	1.00				
GSL-1	0.89	0.78	0.89	1.00			
RSPN-25	0.88	1.00	0.88	0.78	1.00		
HNS-0901	0.88	0.75	0.67	0.78	0.75	1.00	
GSC-101	0.75	0.86	0.75	0.67	0.86	0.63	1.00

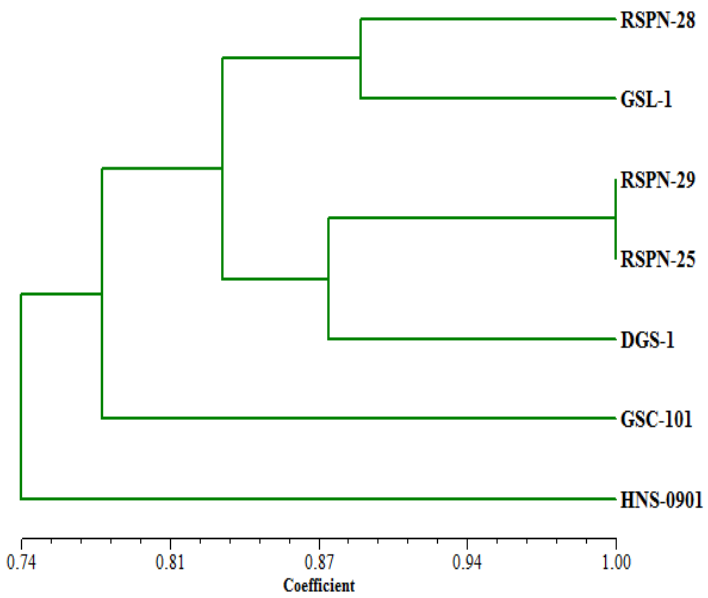

Figure 2. Dendrogram showing the relationships among 7 cultivars of oilseed *Brassica napus* based on SDS-PAGE of seed storage proteins.

Table 4. Cluster pattern of *Brassica napus* genotypes on their genetic divergence.

Protein region	Genotypes/varieties
Class- I	RSN-28 and GSL-1
Class- II	RSPN-29, RSPN-25 and DGS-1
Class- III	GSC-101
Class- IV	HNS0901

Consequently, the region showed both light and dark stained bands. Similar results were found by Turi *et al.,* 2010 and Rabbani *et al.,* 2001 who also reported the same banding pattern in *Brassica species*. Similarly, Kour and Singh (2004) and Nasr *et al.* (2006) obtained the same results.

The results obtained from SDS-PAGE electrophoresis (Figure 1) showed that this method provides a powerful tool for reliable variety identification based on genetic differences in seed storage protein composition among different varieties of *Brassica napus*. Varietal identification was possible in all samples by using SDS-PAGE electrophoresis of seed storage proteins. The genotypes 'GSL-1' showed the highest protein bands (10) followed by 'RSPN-1' and 'DGS-1' (8); 'RSPN-29', 'RSPN-25' and 'HNS-0901' (7) and genotype 'GSC-101' (6). Based on similarity indices, the genotype ranged from 63 to 100%. The highest similarity estimates were recorded in RSPN-25 and RSPN-29, followed by RSPN-28, GSL-1 and DGS-1, GSL-1 (89%). However, the lowest similarity estimates (63%) were recorded in GSC-101 and HNS-0901, followed by 67% in GSL-1 and HNS-0901 (Table 3).

A dendrogram was constructed on the basis of total seed proteins (Figure 2) and classified the varieties into 4 classes. The class -I comprised of 2 genotypes i.e. RSPN-28 and GSL-1; class II includes 3 genotypes (RSPN-29,

RSPN-25 and DGS-1), class III includes the genotype GSC-101 and class IV includes single genotype i.e. HNS-0901. Class II varieties RSPN-29 and RSPN- 25 showed the least genetic distance and consequently have the most genetic linkage. Moreover, class II has the most linkage with class I and the least linkage with three and four classes. Therefore, according to the results in this study, and the result of others, the use of storage seed protein can be recommended for linkage and genetic diversity studies. To study the generic and species level, polyacrylamide gel electrophoresis provides a useful method (Ladizinsky and Hymowitz, 1979) for classical taxonomic classification. However, protein kinds and their differences using SDS-PAGE analysis help the diverse genotypes for breeding programs at seed level and to dig up the record of transparency of genetic resources (Rahman and Hirata, 2004).

From the cluster analysis, it is clear that there is less variation in genotypes. Four clusters and 2 groups were constructed after the analysis of these accessions on a qualitative level (Table 4). The study's finding is further strengthened by the early report of Ghafoor *et al.* (2003), which also concluded the similar results. The results were further strengthened by the earlier findings of Javaid *et al.* (2004) who also reported minimum genetic diversity in groundnut for SDS-PAGE and suggested 2 dimensional (2D) electrophoresis. Turi *et al.* (2010) were also reported similar observations in *Brassica* species. The diverse accessions, having different banding patterns, are suggested to be used in breeding programs. The less divergent may be preserved in the gene bank for use in breeding program (Celis and Bravo, 1984; Beckstrom-Sternberg, 1989).

CONCLUSION

Seed storage protein profiled could be useful marker for genotype identification and diversity analysis (between and within *Brassica* species). Characterization on the basis of proteins and selection of desirable lines/genotypes is great importance for rapeseed breeders. The electrophoresis of seed storage protein is a method to investigate genetic variation. Out of these bands, 5 bands were observed polymorphic. A dendogram was constructed and the genotypes were divided into 2 main groups. The genotypes 'GSL-1' showed the highest protein bands (10) followed by 'RSPN-1' and 'DGS-1' (8); 'RSPN-29', 'RSPN-25' and 'HNS-0901' (7) and genotype 'GSC-101' (6). Based on similarity indices, the genotype ranged from 63 to 100%. The identified diverse genotypes can be used in future breeding programs for the development of variety.

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RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (1) 21-28, 2015

GENETIC DISTANCE, HETEROSIS AND COMBING ABILITY STUDIES IN MAIZE FOR PREDICTING F_1 HYBRID PERFORMANCE

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SUMMARY

Tools for yield prediction are requisite to any successful heterosis breeding program. With this objective present investigation was designed to work out the relationship between genetic diversity, heterosis and specific combining ability (SCA) and yield for predicting the potential F_1 combinations in maize. A field experiment consisting of 10 inbreds of diverse origin and their 45 crosses was conducted for 3 years. Highly significant genotypic variance was observed by joint analysis of variance. It confirmed the sufficient amount of genetic diversity present in parental inbred lines. The estimates of genetic distance based on Euclidean distance matrix and canonical vector analysis showed that B1-12 and B1-15 and B1-12 and B1-34 are most diverse parent combinations. The correlation coefficient analysis showed that there is strong positive association between yield and heterosis and SCA. Genetic distance between parents was also positively correlated with yield and heterosis but weakly associated with magnitude of $r = 0.1059$ and $r = 0.1104$ respectively. However, association between genetic distance and SCA was significantly positive. The findings of this study revealed that higher SCA genetic diversity is important, whereas heterosis and SCA are effective for predicting the best F_1 combinations in maize.

Keywords: Genetic diversity, heterosis, combining ability, correlation, *Zea mays* L.

Short summary statement: Prediction of heterosis through different breeding tools are important to know the inheritance pattern of morphological traits of hybrids along with their parents. This experiment showed that high heterosis for grain yield and other components traits as well as significant SCA effects were most promising combinations for best F_1 in maize which need to be tested on large scale for commercial exploitation of heterosis.

Manuscript received: January 28, 2014; Decision on manuscript: October 6, 2014; Manuscript accepted: October 11, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

Heterosis is a base of breeding program especially for cross pollinated crop like maize. Heterosis depends directly on the existence of dominance and indirectly through interaction involving dominance effect at different loci. According to Falconer and Mackay (1996), the magnitude of heterosis depends on the magnitude of directional dominance and the magnitude of difference in the gene frequency of two parental lines at all the loci affecting the concerned trait. Whereas, the differences in the gene frequency come from diverse genetic back ground of the parents. Thus, it is believed that an important part of heterosis is due to specific

combining ability, and the outstanding single cross combination can be identified only by testing the performance of single crosses. The final evaluation of the inbreds, therefore, consists of the evaluation of single crosses produced from them.

On the other hand, genetic diversity is pre requisite for any crop improvement program, as it helps in the development of superior recombinants (Naik *et al*., 2006). Genetic divergences among the genotypes play an important role in selection of parents having wider variability for different characters. Statistical analysis quantifies the genetically distance among the selected genotype and reflects the relative contribution of specific taints towards the total divergence. The crosses between parents with suitable genetic divergence are generally the most responsive for yielding the most promising segregants, however satisfactory results are obtained only if the germplasm employed in the cross also present high values for the traits of interest (Prasanna, 2012). In most cases, genetic distance is positively correlated with heterosis. Thus, the magnitude of heterosis is generally proportional to the genetic distance between the parents.

The objective of the present study was to evaluate genetic diversity among 10 maize inbred lines to correlate with single cross heterosis, i.e. specific combining ability to the genetic distance of the parental lines for comparison and predicting the most heterotic hybrids.

MATERIALS AND METHODS

Ten maize inbred lines of different origin were selected for these studies (Table 1). All the inbreds were crossed in a diallel fashion without reciprocal crosses. The 45 crosses obtained, together with the 10 parental inbred lines and one local check hybrid (Kanchan 612) were evaluated by randomized block design with 3 replication in 3 environments i.e. summer rainy season of 2010, 2011 and 2012 at Maize Breeding Research Station, SKUAST-J, Poonch (India) located at Latitude 33^0 46' 1.7" N and longitude 74^0 06' 44.1" E with an altitude of 1002 m.

To obtain the estimates of heretosis, combining ability and genetic divergence of the 45 crosses and 10 parental inbred lines, 5 agronomical traits were assessed during the years; grain yield (t ha^{-1}), days to 50% flowering, days to maturity, plant height (cm) and shelling percentage. Data were subjected to analysis of variance for each individual environment (Year), and after conformation of significance for sources of variance, joint analysis of variance was performed. Heterosis values were calculated based on average data from 3 environments as mid-parent (MP) heterosis for grain yield. Estimates of specific combining ability (SCA) for grain yield were evaluated according to method 2 of Griffing (1956) Model I by means of a computer program for diallel analysis (Windostat ver. 8.5).

Genetic divergence among 10 parental inbred lines were determined by Euclidean distance matrix, based on Mahalanobis distance (D^2) using Tocher method described by Rao, (1952). Genetic divergence analysis using canonical (Vector) method was also estimated following the method described by Rao, (1952) by the means of computer program (Windostat ver. 8.5). This is a sort of multivariate analysis, where canonical vectors and PCA scores representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively.

To establish an association between yield, heterosis, SCA and genetic distance among parental lines; correlation coefficient analysis was conducted for identification of most effective breeding technique in predicting the effective heterotic hybrids.

RESULTS

The result of joint analysis of variance showed highly significant genotype mean sum square for all the characters under study. Whereas interaction effect was found significant for grain yield and plant height. (Table 1) The mean grain yield of the crosses ranged 5.17 t ha^{-1} to 10.34 t ha^{-1} and the mean was 7.38 t ha^{-1} (Table 2). The highest heterosis value for grain yield was

Table 1. Information of parent lines and estimation of character effect towards divergence and principal component analysis for individual inbred based on canonical vector analysis**.**

		1 Vector	2 Vector	3 Vector
Eigene Value (Root)		1.974	1.283	0.807
% Var. Exp.		39.487	25.663	16.135
Cum. Var. Exp.		39.487	65.149	81.284
Grain yield kg./ha.		0.151	0.780	0.323
Days to tasselling		0.514	0.453	-0.263
Days to maturity		-0.417	0.264	-0.780
Plant height (cm)		0.515	-0.300	0.011
Shelling %		0.523	-0.164	-0.468
	Genotypes			
Inbred Lines	Origin	Vector 1	Vector 2	Vector 3
1. B1-33	Local germplasm	9.880	3.830	-12.184
2. B1-31	CML 150	11.090	2.990	-11.905
3. B1-32	Local germplasm	10.440	4.850	-11.703
4. B1-34	Local germplasm	9.710	3.480	-11.544
5. B1-11	CML 142	11.030	4.120	-11.710
6. B1-10	CML 149	10.450	4.010	-11.808
7. B1-13	CML 271	11.480	4.710	-11.776
8. B1-12	CML 177	11.880	4.870	-12.285
9. B1-15	CML 141	9.940	2.600	-11.930
10. B1-16	CML 158	11.720	4.280	-12.064

observed from the cross B1-31 x B1-32 (451) followed by B1-11 x B1-12 (418), B1-32 x B1-34 (378) and B1-11 x B1-13 (335) (Table 2). Low heterotic values were also observed for few of the crosses like B1-33 x B1-31 (17.5), B1-33 x B1-13 (36.5), B1-33 x B1-16 (57) and B1-33 x B1-11 (61).

The result of combining ability analysis showed that there was high positive and significant SCA effect for the cross B1-31 x B1-32 (1.62), B1-31x B1-13 (1.56), B1-34 x B1-12 (1.26) and B1-31 x B1-12 (1.20). The highestsignificant negative SCA effect was observed for the cross B1-33 x B1-31 followed by B1-33 x B1-16, B1-34 x B1-10, B1-33 x B1-32 (Table 2).

The estimates of genetic divergence study for genetic distance, ranged from 300.5 for most divergent pair of cross (B1-12 x B1-15) to 45.2 between the least divergent pair (B1-13 x B1-16) (Table 2). The results obtained from canonical vector method for divergence study revealed that the 3 principal components (Vectors) absorbed 39.487, 25.663 and 16.135 percentage of variability, respectively (Table 1). In Z1, highest element value (0.523) was observed for shelling percentage followed by 0.515 for plant height; in Z2 the maximum element value was found for grain yield (0.780) followed by days to tasselling (0.453) and in Z3 days to maturity with element value -0.780 was the highest.

The correlation coefficient between genetic distance and yield, heterosis and specific combining ability were 0.1059, 0.1104 and 0.3520, respectively (Table 3). Whereas the correlation coefficient between yield and heterosis was 0.8676; between yield and SCA was 0.7002 and between SCA and heterosis was 0.6886.

Table 2. Summary of all 45 cross combinations for mean grain yield, heterosis, specific combining ability and genetic diversity between respective parents.

Cross combination	Yield (t ha^{-1})	Heterosis	SCA	Genetic distance* between parents of the cross
B1-33 x B1-31	5.62	17.5	-4.12	145.1
B1-33 x B1-32	6.47	165.5	-2.14	125.4
B1-33 x B1-34	6.41	171.5	-0.78	54.2
B1-33 x B1-11	5.87	61	-0.87	100.9
B1-33 x B1-10	6.06	120.5	-1.13	70.1
B1-33 x B1-13	5.42	36.5	-0.73	178.2
B1-33 x B1-12	6.71	171	0.45	225.4
B1-33 x B1-15	6.73	194	-0.71	107.5
B1-33 x B1-16	5.17	57	-3.41	190.1
B1-31 x B1-32	10.34	451	1.62	206.2
B1-31 x B1-34	8.62	306	1.13	161.1
B1-31 x B1-11	9.06	293.5	0.82	119.3
B1-31 x B1-10	8.76	304	0.61	135.1
B1-31 x B1-13	8.88	296	1.56	183.1
B1-31 x B1-12	7.92	205.5	1.2	200.3
B1-31 x B1-15	8.26	260.5	1.1	121.2
B1-31 x B1-16	8.17	263.5	0.41	145.3
B1-32 x B1-34	8.86	378	0.91	209.1
B1-32 x B1-11	7.29	164.5	-0.44	95.2
B1-32 x B1-10	7.13	189	-1.27	92.6
B1-32 x B1-13	8.66	322	0.47	110.3
B1-32 x B1-12	6.79	140.5	-2.05	129.1
B1-32 x B1-15	7.36	218.5	0.97	210.7
B1-32 x B1-16	7.55	251.5	-0.121	140.4
B1-34 x B1-11	6.44	106.5	-0.36	150.4
B1-34 x B1-10	5.9	93	-2.16	112.1
B1-34 x B1-13	6.17	100	1.11	220.4
B1-34 x B1-12	6.82	170.5	1.26	250.1
B1-34 x B1-15	6.23	132.5	-1.34	90.2
B1-34 x B1-16	5.88	111.5	-0.63	117.4
B1-11 x B1-10	8.69	315.5	0.46	70.2
B1-11 x B1-13	9.09	335.5	0.23	80.6
B1-11 x B1-12	9.86	418	1.06	111.3
B1-11 x B1-15	8.81	334	0.45	188.2
B1-11 x B1-16	8.43	310	0.11	70.8
B1-10 x B1-13	7.02	169	-0.76	120.6
B1-10 x B1-12	7.11	183.5	-2.6	158.4
B1-10 x B1-15	6.87	180.5	-0.18	150.7
B1-10 x B1-16	7.07	214.5	-0.14	130.3
B1-13 x B1-12	6.88	140.5	-1.15	65.4
B1-13 x B1-15	7.54	227.5	0.32	264.2
B1-13 x B1-16	7.16	203.5	-0.33	45.2
B1-12 x B1-15	7.14	193	0.27	300.5
B1-12 x B1-16	6.82	175	-1.92	60.2
B1-15 x B1-16	8.06	320	0.79	242.1

*Genetic distance between respective inbred combinations for 5 morphological characters based on Mahalanobis distance (D^2)

Table 3. Correlation coefficient between grain yield, specific combining ability, heterosis and genetic distance.

	Yield	Heterosis	SCA	Genetic Distance (D^2)
Yield	1.0000			
Heterosis	0.8676**	1.0000		
SCA	0.7002**	0.6886**	1.0000	
Genetic Distance (D^2)	0.1059	0.1104	0.3520**	1.0000

** Significant at the 0.05, i.e. 0.01 probability level.

Table 4. Summary of joint analysis of variance for 5 different morphological characters of all 45 cross combination.

	Mean sum square				
Trait	Genotype	Genotype x Environment	Error	Mean	CV (%)
Grain yield (t ha^{-1})	4814.73**	2252.01*	760.07	7.38	15.6
Days to tasselling	31.95**	3.01^{ns}	2.29	55.9	4.3
Days to maturity	28.84**	3.62^{ns}	7.31	120.7	3.5
Plant height (cm)	647.75**	212.13*	113.73	244.9	6.9
Shelling %	9.43*	5.12^{ns}	4.40	85.5	3.9

*, ** Significant at the 0.05, i.e. 0.01 probability level, respectively.

DISCUSSION

The combined analysis of variance across environments showed maximum grain yield variation due to genotypes, confirming the presence of high degree of genetic diversity in breeding material. Whereas, environment also played significant role in variation for grain yield, reflected in significant interaction effect (Table 4). Eighteen F_1 hybrids showed highest result for grain yield. The grain yield has become the main focus of breeding as it is connected to other characters (Rahman *et al.,* 2007).

The values of heterosis for grain yield were high positive for most of the crosses indicating the absence of bidirectional dominance derivatives. The parents of highly heterotic cross (B1-31 x B1-32) had different genetical background i.e. local germplasm and CIMMYT breeding line. Hallauer and Miranda (1981) explained that in addition to the existence of genes with some degree of dominance controlling the character, the expression of heterosis also depends on the divergence between genotypes, as differences in allele frequencies are required at loci involved in the expression of desirable characteristics. Whereas low heterotic effect is likely due to low genetic complementarily of loci with non-additive effects, possibly because these crosses displayed some degree of parental relationship (Aliu *et al.,* 2008).

Estimates of specific combining ability for predicting the yielding capacities of the crosses produced, indicated the presence of positive and negative cross compatible parents in the population of study with high and low magnitude of interaction effects (Table 2). Sundarajan and Kumar (2011) and Makumbi *et al.,* (2012) also observed the different level of SCA effects in his experiments. The high positive and significant SCA effect highlighted the importance of non-additive (dominance and/or epistatic) gene action expressed in F_1 cross that performed better than the mean of their parents. However, there were crosses with highly negative SCA values like. B1-33 x B1-31. The influence of SCA could bring about both positive and negative values. Positive value meant that F_1 hybrid was better than F_1 hybrid that having negative value (on the equivalent character) (Muraya *et al*., 2006; Gowda *et al*., 2013). The cross with high positive SCA effect (B1-31 x B1-32) also showed the high heterotic effect in the present study. These data agree with

the findings of Devi and Singh (2011) who stated that SCA and heterosis are highly related parameters.

Genetic divergence study of parental lines through Euclidean distance matrix based on Mahalanobis D^2 revealed that there was wide range of genetic diversity from 300.5 to 45.2. These data are supported by the findings of Li *et al.* (2004) and Rodrigo *et al.* (2012). Showemimo (2004) reported that estimates of the generalized Mahalanobis distance (D2) clearly indicated that the pairs of genotypes are more divergent and more similar genetically.

With the aim of more critical prediction of genetic divergence between genotypes, multivariate methods, such as principal component analysis and canonical variables were applied in the present study. The result showed that the first principal component (vector I) absorbed and accounted for maximum (39.48%) proportion of variability and remaining once accounted for progressively lesser and lesser amount of variation (25.63% and 16.13%) for vector II and vector III, respectively (Table 1). The highest element value (0.523) was observed for shelling % in vector I (PCA I), whereas, the maximum element value (0.780) was recorded for grain yield in vector II (PCAII). As the first 2 vectors showed eigen values more than one and cumulatively they expressed 65.15% variability (Table 1). Genetic divergence between genotypes measured in terms of spatial distance and resulted in formation of 2 dimensional (2D) representation based on 2 PCA scores (vector I and vector II) for each individual genotypes (Figure 1). The figure reflected highest diversity between B1-34 and B1-12 followed by between B1-12 and B1-15. Whereas, minimum diversity were observed between B1-33 and B1-34 followed by between B1-13 and B1-16. The estimates of genetic divergence based on PCA score method was slightly differ from Euclidean distance matrix. These findings are in general agreement with the findings of Dauda and Olakojo (2007) and Alam *et al*. (2012).

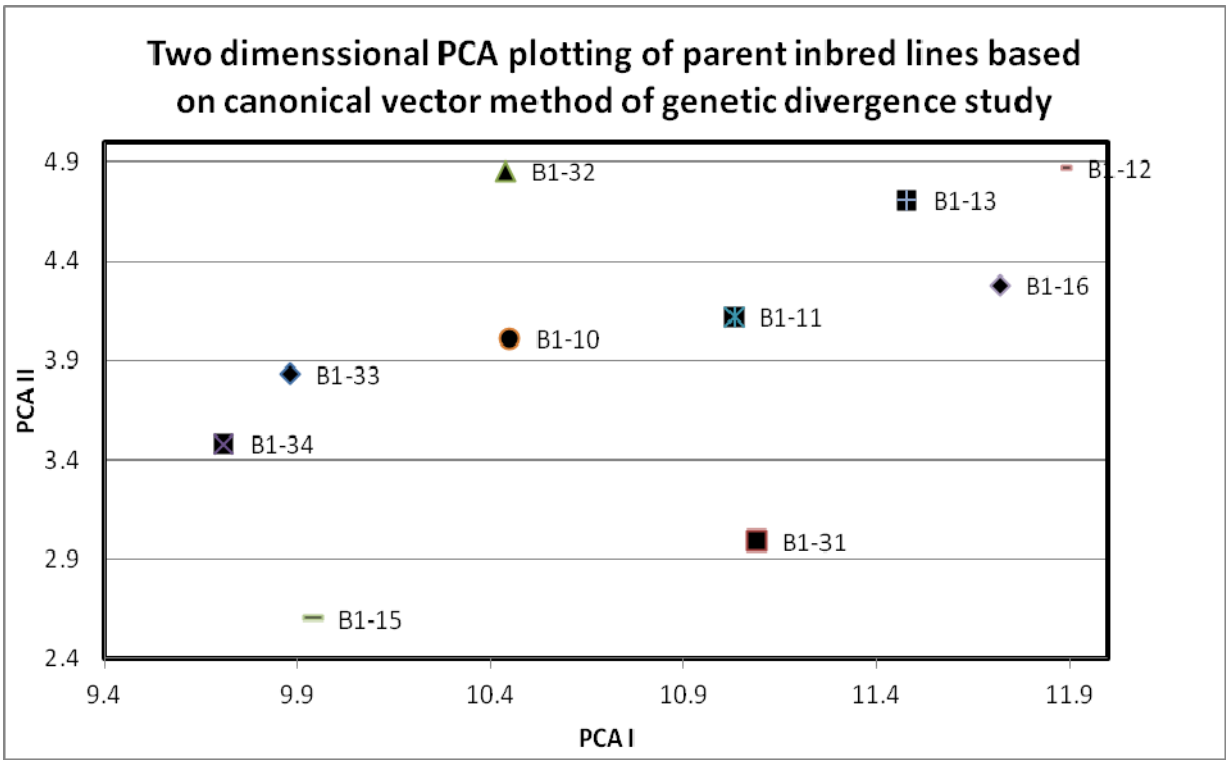

Figure 1. 2D representation of inbred lines for their genetic divergence.

Results presented in Table 3 are demonstrating positive correlation between parental genetic distance and F_1 yield, specific combining ability and heterosis. The correlation coefficient between genetic distance and grain yield for hybrids is positive but weak (r = 0.1059). The correlation coefficient of genetic distance and specific combining ability (SCA) were positive and significant (r = 0.3520). The result also indicated that the correlation between genetic distance of parents and heterosis for yield of hybrids is positive but weak (r = 0.1104) (Wegary *et al.*, 2013). This is supported by the findings of Drinic *et al.* (2002), Marsan *et al.* (1998) and Betran *et al.* (2003). The cross between the most divergent inbreds (B1-12 x B1-15) (Table 2) showed heterosis of 193. Moreover, the cross between least divergent parents (B1-11 x B1-13) represented heterosis of 335.5. These data indicated that estimate of genetic diversity alone may not be enough to reveal the best combination of genotypes for successful breeding program.

On the other hand, strong positive and significant correlation was observed between yield and heterosis and SCA i.e. r = 0.8676 and r = 0.7002, respectively. The correlation between heterosis and SCA of crosses were also found to be strong, positive and significant (Drinic *et al.*, 2002 and Betran, *et al.*, 2003). The relationship between grain yield and heterosis and specific combing ability could be used to predict F_1 hybrid appearance (Devi and Singh, 2011).

Genetic diversity is necessary for heterosis as it is positively related with yield. However, it is not sufficient to predict the performance of F_1 combinations. Hence, estimation of heterosis and combining ability together provide effective breeding tool to predict the yield potential of maize F_1 hybrids.

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RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (1) 29-39, 2015

EVALUATION OF CYTOPLASMIC MALE STERILE (CMS) PROGENIES AND MAINTAINER LINES FOR YIELD AND HORTICULTURAL TRAITS IN CABBAGE (*Brassica oleracea* var. *capitata* L).

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SUMMARY

Cabbage, *Brassica oleracea* var. *capitata* L. is one of the most important cole group vegetables. Hybrids are known to outperform the open-pollinated varieties in marketable head yield and horticultural traits. Cytoplasmic male sterility (CMS) seems to be a better option over sporophytic self-incompatibility (SSI) to develop hybrids in cole crops because of the standard advantages. Hence, the present investigation was undertaken to compare the CMS progenies with their respective fertile counterparts (maintainer lines) for yield and horticultural attributes. The experimental material was evaluated in RBD with 3 replications at the Experimental Farm of the Department of Vegetable Science and Floriculture, CSKHPKV, Palampur, Distt. Kangra H.P during 2010-11 and 2011-12. Observations were recorded on yield and important horticultural traits. The CMS progenies were also evaluated for their sterility behavior during the flowering regimes *viz*., 25-50%, 50-75% and 75-100%. All the 4 CMS progenies had the head shape index at par with their respective maintainer lines. For days to harvest, except KGAT- I CMS during 2011-12 the CMS progenies were at par with their respective fertile counterparts as well. For the traits heading (%), marketable heads per plot, non wrapper leaves and gross weight, one or two of the CMS progenies were not at par with respective maintainer lines. In respect of compactness of heads, net weight of heads and marketable head yield (kg/plot), the exceptions were even up to 50% of the CMS progenies studied. This may be attributed to the facts that cabbage is a highly cross-pollinated vegetable and the expression of latter traits subject to the influence of prevailing weather conditions. All the CMS progenies revealed break- down of male sterility (very mild to mild pollen grain production) in variable number of plants in all the flowering regimes during both the years (2010-11 and 2011-12). There exists a possibility of developing stable CMS progenies through rigorous selection over the years.

Keywords: Cabbage, cytoplasmic male sterility, stability, yield, flowering regimes, horticulture traits

Short summary statement: Development and standardizing protocol through CMS for HSP in cabbage is required urgently for large and good quality seed production of hybrids. Useful information has been generated especially in low chilling type of cabbage genotypes and the lines will be useful for the breeders for scaling up their HSP in this crop with ease. Hence, the present research work will have an impact and contribution on research in this field.

Manuscript received: July 10, 2014; Decision on manuscript: October 6, 2014; Manuscript accepted: January 3, 2015.

Communicating Editor: Bertrand Collard

INTRODUCTION

Cabbage, *Brassica oleracea* var. *capitata* L. (2n = 2x = 18), a member of family *Brassicaceae*, is one of the most important crops of the cole group of vegetables. Closely related to other cole crops, such as broccoli, cauliflower and brussels sprouts, it has originated from *Brassica oleracea* var. *oleracea* L. (syn. *sylvestris* L.) commonly known as wild cabbage through mutation, human selection and adaptation. Most common cruciferous vegetables eaten by people, known colloquially as cole crops (cabbage, cauliflower, knolkhol, brussels sprout, kale and broccoli) are in a single species (*B. oleracea*); they are not distinguished from one another taxonomically, only by horticultural category of cultivar groups. Cabbage (*B. oleracea* var. *capitata*) is considered to be a typical representative of the C genome of *Brassica*. It is a rich source of vitamins A, B, C and minerals like phosphorus, potassium, sodium and iron. In India it ranks next to cauliflower with acreage and first with respect to production among cole crops occupying an area of 3,72,000 ha with annual production of 8534,000 tons (Anonymous, 2013).

Hybrids are known to outperform the open-pollinated varieties in marketable head yield and horticultural traits. The genetic phenomenon of sporophytic self-incompatibility (SSI) and cytoplasmic male sterility (CMS) is being used to develop cabbage hybrids on commercial scale (Parkash, 2008). With the successful transfer of R-cytoplasm (Ogura) induced CMS system in cole crops (including cabbage), there has been a lot of interest in its use for hybrid development during the last over a decade now (Melo and Giordano, 1994). Compared with self-incompatible lines, cytoplasmic male sterile lines could increase cultivars purity in cabbage hybrid by 5-7% (Ding and Jian, 2008). Self-incompatibility is not always stable, and may be suppressed by high temperature or drought and CMS (Cytoplasmic male sterile) lines are stable over the range of environments (Yamagishi and Bhat, 2014). Cytoplasmic male sterility seems to be a better option to develop hybrids in cole crops. In India, the hybrids of cabbage developed in public sector through the use of CMS are KTCBH-84 and H-64 (Anonymous, 2011). This is therefore considered as an seen as an alternative approach overcoming the problems (self in hybrid seed due to breakdown of self incompatibility and lengthy process of development of homozygous SI lines) experienced while using sporophytic self-incompatibility system.

At CSKHPKV, Palampur, the incorporation of cytoplasmic male sterility into low chill requiring genotypes of cabbage had been started in 2003-04. The CMS progenies are now in BC_6 stage quite comparable with their male fertile counterparts. Therefore, considering the importance of cytoplasmic male sterility in the production of hybrid seed in cabbage, present studies were carried out to evaluate the CMS progenies along with their maintainer lines for yield and horticultural traits and to evaluate CMS progenies for their stability in different flowering regimes.

MATERIALS AND METHODS

The investigation was undertaken at the Experimental Farm of Department of Vegetable Science and Floriculture, CSKHPKV, Palampur situated at situated at 32^0 6' N latitude and 76^0 3' E longitudes at an elevation of 1290.8 m above mean sea level, during 2010-2012. During 2010-11, the seeds of 4 CMS lines and their maintainers (Table 1) were sown in the nursery on 9th September, 2010 (inside polyhouse) and transplanting of seedlings was carried out on 21st October, 2010 at 60 x 45 cm spacing in open field following Randomized Complete Block Design (RCBD) with 3 replications. Each experimental plot was of the size 3.0 x 2.7 m accommodating 30 plants. During 2011-12, the seeds of respective CMS and maintainer lines were sown in the nursery on 7th September, 2011 (inside polyhouse) and transplanting was carried out on 15th October, 2011. The experimental design, replication, plot size and spacing were similar to that of 2010-11 seasons.

All the recommended package of practices was followed to ensure the proper growth of plants. The observations on the plant characters namely days to harvest, gross weight (g), number of non wrapper leaves, net weight of

Table1. List of CMS progenies and their male fertile counterparts used in the study.

Genotypes	Source	Remarks
KGAT-I CMS	CSK HPKV, Palampur	male sterile
KGAT-II CMS	CSK HPKV, Palampur	male sterile
KGAT-III CMS	CSK HPKV, Palampur	male sterile
GA(P) CMS	CSK HPKV, Palampur	male sterile
KGAT- I	CSK HPKV, Palampur	male fertile
KGAT- II	CSK HPKV, Palampur	male fertile
KGAT- III	CSK HPKV, Palampur	male fertile
GA(P)	CSK HPKV, Palampur	male fertile

head (g), polar diameter of head (cm), equatorial diameter of head (cm), head shape index, compactness of head, marketable heads per plot (No.), heading(%) and marketable head yield (kg/plot) were recorded in each treatment in each replication. Except heading (%), marketable heads and marketable head yield per plot, the observations were recorded on 10 plants taken at random in each treatment/plot. The shape index of head was calculated by dividing the polar diameter with equatorial diameter (Odland and Noll, 1954). Compactness of head was determined as per the procedure suggested by Pearson (1931). For the stability of CMS progenies, 10 plants per treatment/plot/replication were retained at random for bolting and flowering and were observed for sterility during 3 flowering regimes i.e 25-50% flowering, 50-75% flowering and > 75% flowering. The experimental plots/plants of replication-I were covered with UV stabilized insect-proof nylon-nets during all the flowering regimes on bamboo frame-work. The flowering branches in replication-II and replication-III were covered with butter paper bags of the size 26.0 x 10.0 x 5.0 cm.

Statistical analysis

Average values/plant or plot for each genotype in each replication for the traits studied were subjected to statistical analysis. The statistical analysis of experimental data was accomplished by Analysis of Variance in randomized block design (RBD) as per the procedure given by Panse and Sukhatme (1985) using CPCS-1 software (Cheema and Singh, 1990).

RESULTS AND DISCUSSION

Analysis of variance for the experimental data (Table 2) revealed that mean squares due to genetic stocks (treatments) were significant for all the traits studied. The genotype KGAT- I CMS was the earliest to produce marketable heads in 115.1 and 110.7 days during 2010-11 and 2011-12 respectively. The genotype KGAT-III took the maximum days of 128 and 130 during 2010-11 and 2011-12 respectively. Except the progeny KGAT- I CMS during 2011-12, all the remaining 3 CMS progenies were at par with their respective maintainer lines during both the years. This implies that the CMS lines are by and large as good as maintainer lines except for being male sterile. The mean values of gross weight are presented in Table 3. The highest and the lowest gross weight/plant were recorded in KGAT- III CMS (1235 g) and GA(P) (938.3 g) in 2010 -11 and in KGAT- I CMS (999.6 g) and KGAT- III (786 g) during 2011-12. Except the progenies KGAT- III CMS and GA(P) CMS during 2010-11 and KGAT- III CMS during 2011-12, all the remaining CMS progenies were at par with their maintainer lines. The gross weight of CMS progenies KGAT- II CMS, KGAT- III CMS and GA(P) CMS were higher than that of respective male fertile maintainer during 2010-11. It is possible that these CMS progenies might not have turned isogenic even after BC_6 generation for this trait.

Table 2. Analysis of variance with respect to 11 traits studied during 2010-12.

Character	Source	Replications		Genotypes		Error	
	Degrees of freedom	2		7		14	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Days to harvest		78.31*	346.73*	49.05*	109.37*	16.87	11.02
Gross weight(g)		112011.0*	29390.0*	31328.0*	15402.38*	3827.85	1837.24
Number of non wrapper leaves		1.17	8.04*	11.50*	11.49*	1.50	0.51
Net weight of head(g)		58650.0*	6650.25*	10536.8*	14219.19*	891.59	637.72
Polar diameter of head(cm)		2.95*	2.26*	0.75*	1.16*	0.12	0.06
Equatorial diameter of head(cm)		7.80*	4.62*	0.77*	0.81*	0.14	0.20
Head shape index		0.006*	0.02*	0.01*	0.008*	0.001	0.001
Head compactness		897.96*	142.31*	44.52*	54.98*	10.40	8.78
Marketable heads per plot (No.)		0.405*	0.25*	0.359*	0.25*	0.05	0.04
Heading (%)		28.79*	12.87*	23.61*	10.99*	1.93	1.77
Marketable head yield (kg/plot)		13.77*	8.40*	23.21*	10.69*	0.77	0.35

Table 3. Days to harvest (No.) and gross weight (g/plant) in cytoplasmic male sterile (CMS) and maintainer lines of cabbage.

Genotype	Days to harvest		Gross weight/plant	
	2010-11	2011-12	2010-11	2011-12
KGAT- I CMS	115	111	1183	999
KGAT- II CMS	120	119	1208	865
KGAT- III CMS	124	127	1235	875
GA(P) CMS	118	124	1093	946
KGAT- I	122	120	1197	971
KGAT- II	119	121	1207	904
KGAT- III	128	130	1056	786
GA(P)	120	126	938	977
C.D. (5%)	7.2	5.8	108.3	75.1
C.V. (%)	3.4	2.7	5.4	4.5

*Values in table are round off to whole number

Alternatively, this may be attributed to the fact that in CMS progenies we had taken a random sample of seeds harvested from true male sterile plants whereas in case of maintainer lines we had taken a random sample from the seeds harvested on all the plants.

Days to harvest indicate the maturity of a given genotype. The mean values for days to harvest after transplanting are presented in Table 3. The mean values of non-wrapper leaves, net weight of head, polar and equatorial diameter of head are presented in Table 4. The genotype KGAT- I recorded the maximum number of non wrapper leaves 17 and 16 in 2010-11 and 2011-12 respectively. On the other hand, GA(P) CMS recorded the minimum number of non-wrapper leaves 11 and 10.6 in 2010-11 and 2011-12 respectively. KGAT- II CMS and KGAT- III CMS were at par with their maintainer lines during both the years. However, GA(P) CMS was at par to its maintainer line in 2011-12 only. The net weight of head is a direct component of marketable head yield. The highest and the lowest net head weight/plant were recorded in KGAT- III CMS (660 g) and KGAT- III (475 g) in 2010-11 and in GA(P) (606 g) and KGAT- III (432 g) during 2011-12 respectively. The progenies KGAT- I CMS and KGAT- II CMS were at par with their respective maintainers in 2010-11 whereas KGAT- III CMS and GA(P) CMS in 2011-12 were at par with their respective maintainer lines. This may be attributed to seasonal/climatic variations. Polar diameter of head is one of the 2 traits which govern the head shape.

During 2010-11, the highest and lowest values for polar diameter were recorded in GA(P) (12 cm) and KGAT- II CMS (10 cm) respectively, whereas in 2011-12, KGAT- I (12) as well as GA(P) (12 cm) recorded the highest whereas KGAT- III CMS (10 cm) had the lowest polar diameter. However, KGAT- III CMS and KGAT- II CMS were at par with each other during 2011-12. Except the progeny KGAT- III CMS in 2010-11 and KGAT- I CMS in 2011-12, all the other CMS progenies were at par with their maintainer lines. The perusal of mean values (Table 4) revealed that in 2010-11 both KGAT- III CMS (12 cm) as well as KGAT- II (12 cm) recorded the maximum equatorial diameter whereas in 2011-12, KGAT- II had the highest value (13 cm) but this was at par with KGAT- III CMS (11 cm). During 2010-11, the lowest equatorial diameter (11 cm) was in the treatment GA(P) CMS but during 2011-12, this was in the treatment KGAT- III CMS (11 cm) which was at par with GA(P) CMS.

Head shape index value (polar: equatorial diameter) reflects the shape of cabbage heads. In case of a normal head (spherical) the head shape index value is between 0.8-1.0. The head shape index values below 0.8 indicate flat or drumhead type heads whereas the values > 1.0 indicate pointed heads. The ratio of polar and equatorial diameter was used to work out the head shape index (Table 5). This ranged between 0.8 in KGAT- II CMS to 1.0 in GA(P) and GA(P) CMS during 2010-11 and 0.8 in KGAT- II CMS and 0.9 in GA(P) CMS during 2011-12. All the 4 CMS progenies were at par with their maintainer lines indicating similarity in head shape. Since the head shape index values are between 0.8 and 1.00, all the CMS progenies as well as maintainer lines fall in the category of normal (spherical) heads which are acceptable to the consumers.

Head compactness is a desirable attribute in the sense that more produce can be accommodated in lesser shape/volume. The perusal of mean values (Table 5) revealed that head compactness varied from 34.8 in GA(P) to 46.1 in KGAT- II CMS during 2010-11 whereas the range was from 24.2 in KGAT-I to 37.5 in GA(P) CMS during 2011-12. In general, the head compactness (z) values were higher during 2010-11 as compared to 2011-12 indicating the effect of season/climate on the expression of this character. Except the CMS progenies KGAT- II CMS and GA(P) CMS during 2010-11 and KGAT- I CMS 2011-12, all the remaining CMS progenies were at par with the maintainer lines.

Marketable heads per plot contribute directly towards marketable yield. The mean values for number of marketable heads per plot have been presented in Table 5.

Table 4. Number of non-wrapper leaves and net weight of head (g)/plant, polar diameter of head (cm) and equatorial diameter of head (cm)/plant in CMS and maintainer lines of cabbage.

Genotype	No of non wrapper leaves		Net weight of head /plant		Polar diameter of head (cm)		Equatorial diameter of head	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
KGAT- I CMS	13	13	630	551	11	11	12	12
KGAT- II CMS	14	14	611	450	10	11	12	13
KGAT-III CMS	12	12	660	443	11	10	12	11
GA(P) CMS	11	11	610	575	11	12	11	12
KGAT- I	17	16	588	445	10	12	11	13
KGAT- II	15	13	597	515	11	11	12	13
KGAT- III	13	12	475	432	10	11	11	12
GA(P)	12	12	527	606	12	12	12	12
C.D. (5%)	1.3	1.2	52.3	44.3	0.6	0.4	0.7	0.8
C.V. (%)	5.4	5.3	5.0	5.0	3.3	2.4	3.3	3.7

Table 5. Head shape index, head compactness and marketable heads/plot in CMS and maintainer lines of cabbage.

Genotype	Head shape index		Head compactness (Z value)		Marketable heads /plot	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
KGAT- I CMS	0.9	0.9	43.9	34.6	26.7	29.3
KGAT- II CMS	0.8	0.8	46.0	29.7	27.0	28.0
KGAT-III CMS	0.9	0.9	41.8	35.3	26.3	28.0
GA(P) CMS	1.0	0.9	44.2	37.4	21.6	27.3
KGAT- I	0.9	0.9	44.7	24.2	27.0	30.0
KGAT- II	0.8	0.8	38.6	31.8	27.0	28.6
KGAT- III	0.9	0.9	38.7	31.5	20.6	25.0
GA(P)	1.0	0.9	34.8	36.3	22.0	24.6
C.D. (5%)	0.1	0.07	5.6	5.2	2.4	2.3
C.V. (%)	4.0	4.7	7.7	9.1	5.6	4.8

*Values in tables are round off to 1 decimal point only.

During 2010-11, KGAT- II CMS, KGAT- I and KGAT- II recorded the maximum (27) number of heads/plot whereas KGAT- III produced the minimum (20.6) marketable heads per plot.

The data for heading (%) have been presented in Table 6. During 2010-11, KGAT- II CMS (97.3%) and KGAT- III (76 %) and during 2011-12, KGAT- I (100 %) and GA(P) (82.6 %) recorded the highest and the lowest heading (%) respectively. Except KGAT- III CMS during 2010-11 and GA(P) CMS during 2011-12, all the remaining CMS progenies were at par with their maintainer lines.

Marketable head yield is the dependent variable which is of economic concern/importance to the breeders and farmers. The perusal of mean values (Table 6) revealed that the genotype KGAT- III CMS (17.3 kg) and KGAT- III (9.8 kg) in 2010-11 and the genotype KGAT- I CMS (16.1 kg) and KGAT- III (10.8 kg) in 2011-12 recorded the maximum and the minimum head yield (kg/plot) as well as q/ha respectively. In the year 2010-11, KGAT- I CMS and KGAT- II CMS were at par with their respective maintainer lines whereas in 2011-12, GA(P) CMS was the only progeny which was at par with its maintainer line. The CMS progenies and the maintainer lines did not reveal a definite trend in marketable head yield (kg/plot or q/ha) probably due to variation in climatic conditions during the period of the present investigation. As per the mathematical expectations of backcross method, the BC_6 progenies should behave isogenic to the recurrent parent. Jian and Ding (2005) had also developed cytoplasmic male sterile lines in cabbage through backcrossing of inbred lines in cabbage for 5-6 generations and they had been successful in getting perfect hybrids with uniform and good characters. Chen *et al.* (1995) had also developed cytoplasmic male sterile lines of Indian mustard which were almost equal to the maintainer lines and standard varieties in economic traits.

In this study, all the four CMS progenies had the head shape index at par with their respective maintainer lines. For days to harvest, the exception was KGAT- I CMS during 2011-12 only. For the traits heading (%), marketable heads per plot, non-wrapper leaves and gross weight, one or two of the CMS progenies were not at par with respective maintainer lines. In respect of the traits compactness of heads, net weight of head and marketable head yield (kg/plot), the exceptions were even up to 50 % of the CMS progenies studied. This may be attributed to the facts that cabbage is a highly cross-pollinated vegetable crop and the latter traits are prone to the influence of prevailing weather conditions.

Table 6. Heading percentage, marketable head yield (kg/plot and q/plot) in CMS and maintainer lines of cabbage.

Genotype	Heading %		Marketable head yield		Marketable head yield	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
KGAT- I CMS	92.3 (9.6)	97.3 (9.8)	16.9	16.1	209.3	198.9
KGAT- II CMS	97.3 (9.8)	94.0 (9.7)	16.5	12.6	203.6	155.1
KGAT-III CMS	94.6 (9.7)	94.0 (9.7)	17.3	12.3	213.9	152.8
GA(P) CMS	87.3 (9.3)	92.6 (9.6)	13.4	15.7	164.8	194.3
KGAT- I	90.6 (9.5)	100 (10)	15.9	13.3	196.4	164.7
KGAT- II	92.0 (9.6)	95.0 (9.7)	16.1	14.7	198.4	182.1
KGAT- III	76.0 (8.7)	87.6 (9.3)	9.8	10.8	120.9	133.5
GA(P)	89.6 (9.4)	82.6 (9.1)	11.6	15.4	143.0	189.1
C.D. (5%)	0.4	0.4	1.5	1.0	18.8	12.7
C.V. (%)	2.4	2.3	5.9	4.2	5.9	4.2

Values in the parentheses are square root transformations.
Values are round off to 1 decimal point only.

Stability of male sterility in cytoplasmic male sterile progenies

Ten plants per CMS progeny per replication were observed for stability of male sterility in the CMS progenies during the flowering regimes 25-50%, 50-75% and >75% flowering and the data are presented in Table 7. During 2010-11, the number of plants showing the production of very mild to mild pollen grains ranged from 0 (KGAT- III CMS) to 9 in GA(P) CMS in the flowering regime 25-50%. It ranged from 3 in KGAT- II CMS and KGAT- III CMS to 9 in GA(P) CMS and KGAT- I CMS in the flowering regime 50-75%. The range was from 3 in KGAT- II CMS to 9 in GA(P) CMS and KGAT- I CMS during the flowering regime 75-100%. During 2011-12, the number of plants showing the production of very mild to mild pollen was relatively lesser. The range was 0 in KGAT- I CMS and KGAT- III CMS to 1 in KGAT- II CMS and GA(P) CMS in the flowering regime 25-50%, 2 in KGAT- III CMS to 4 in KGAT- II CMS in the flowering regime 50-75% and 4 in KGAT- IIICMS and GA(P)CMS to 6 in KGAT- I CMS in the flowering regime 75-100%.

In order to ensure whether the pollen grains produced on otherwise male sterile plants were fertile or not, these were subjected to acetocarmine staining test (Chandrashekra *et al.*, 2013) along with maintainer lines producing abundant pollen grains during the year 2010-11. The average numbers of stained pollen grains are given in Table 8.

All the CMS progenies showed stained pollen grains which were found in numbers ranging from 54.7 in KGAT- III CMS to 104.3 in KGAT- ICMS but these were slightly lower in number as compared to their respective maintainer lines.

The number of plants showing the production of very mild to mild pollen grains during 2011-12 was lower in the CMS progenies KGAT- I CMS and GA(P) CMS constant in KGAT- III CMS and more in KGAT- II CMS. This indicates that there is a possibility of reducing the breakdown of male sterility in the CMS progenies through rigorous selection over the years.

Table 7. Number of plants showing stability and breakdown of male sterility in different flowering regimes in the CMS progenies of cabbage during 2010-11 and 2011-12.

S.N0.	CMS progenies	Flowering regimes						Stable plants(%) in CMS progenies	Breakdown of sterility (%)	Pollen category	Plant mortality due to stalk rot (No.)
		25-50%		50-75%		75-100%					
		Stable	breakdown	stable	breakdown	stable	breakdown				
2010-11											
1	KGAT- I CMS	27	3	21	9	21	9	70.0	30.00	Very mild-mild	0
2	KGAT- II CMS	28	2	27	3	27	3	90.0	10.00	Very mild-mild	0
3	KGAT- III CMS	30	0	27	3	26	4	86.7	13.33	Very mild-mild	0
4	GA(P) CMS	18	9	18	9	18	9	66.7	33.33	Very mild	3
2011-12											
1	KGAT- I CMS	29	0	26	3	23	6	79.3	20.68	Very mild-mild	1
2	KGAT- II CMS	28	1	25	4	24	5	82.7	17.24	Very mild-mild	1
3	KGAT- III CMS	30	0	28	2	26	4	86.6	13.33	Very mild-mild	0
4	GA(P) CMS	29	1	27	3	26	4	86.6	13.33	Very mild-good	0

* All the plants in the maintainer lines KGAT-I, KGAT-II, KGAT- III and GA(P) were male fertile.

Table 8. Stained pollen grain count of male sterile plants contributing very mild to mild pollen grains and the fertile maintainer lines during 2010-11.

Genotype	Average number of stained pollen grains/count
KGAT- I CMS	104.3
KGAT- II CMS	64.4
KGAT- III CMS	54.7
GA(P) CMS	79.8
KGAT- I	134.5
KGAT- II	100.9
KGAT- III	88.5
GA(P)	89.6

All the 4 CMS progenies had the head shape index at par with their respective maintainer lines. For days to harvest, except KGAT- I CMS during 2011-12, the CMS progenies were at par with their respective fertile counterparts as well. For the traits heading (%), marketable heads per plot, non-wrapper leaves and gross weight per plant, 1 or 2 of the CMS progenies were not at par with respective maintainer lines. In respect of the traits compactness of heads, net weight of head and marketable head yield (kg/plot), the exceptions were even up to 50% of the CMS progenies studied. This may be attributed to the facts that cabbage is a highly cross-pollinated vegetable crop and the latter traits are subject to the influence of prevailing weather conditions.

All the CMS progenies revealed breakdown of male sterility (very mild to mild pollen grain production) in variable number of plants in all the flowering regimes during both the years (2010-11 and 2011-12). The number of plants showing the production of very mild to mild pollen grains during 2011-12 were lower in the CMS progenies KGAT-I CMS and GA(P) CMS, constant in KGAT- III CMS and more in KGAT- II CMS. This suggests the possibility of developing stable CMS progenies through rigorous selection over the years. Alternatively, it will be a desirable proposition to study the impact of very mild – mild pollen grain production in the plants of CMS progenies on the true to type characteristics of the hybrids developed by using such CMS lines.

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RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (1) 40-48, 2015

GENETIC DIVERSITY AND GENOTYPE BY TRAIT ANALYSIS FOR AGRO-MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF WHEAT (*Triticum aestivum* L.)

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SUMMARY

Assessment of genetic diversity and its application for wheat breeding results in enhanced and sustainable production and productivity. Twenty-three wheat genotypes along with 2 checks were evaluated for 9 agro-morphological and physiological traits viz., early vigor, leaf firing, days to heading, plant height, number of tillers/m, chlorophyll content Index (CCI) at 3 stages and grain yield. The data was further subjected to PCA (principal component analysis) and genotype by trait biplot analysis. The first 5 principal components accounted for 90.5% of total variation. The cluster analysis shows that there is significant genetic variability among tested wheat genotypes that indicates the presence of excellent opportunity to bring about improvement through hybridizing the selected genotypes present in distant clusters. Across the 25 tested wheat genotypes grain yield was positively associated with tillers/m and early vigor while negatively associated with leaf firing. CCI (Chlorophyll Content Index) measured at 3 stages, were positively associated with each other. The vector traits yield made a 180 degree angle with leaf firing indicating they were opposite in genotype ranking.

Keywords: Genotype by trait biplot, principal component analysis, wheat

Short summary statement: Winter wheat gene pool is a rich reservoir of genetic diversity for various traits of economic importance. It thus becomes imperative to assess the variability for different traits and utilize it in the wheat breeding programs. This study has identified the winter wheat derived genotypes for agro-morphological and physiological traits and these genotypes can be used in breeding programs.

Manuscript received: August 8, 2014; Decision on manuscript: September 12, 2014; Manuscript accepted: October 3, 2014.

Communicating Editor: Sam Periyannan

INTRODUCTION

Wheat is one of the world's most important agricultural crops; with annual global grain production estimated around 710 million tons (IGC, 2014) and it provides 20% of the calories and protein for the world's population (Braun *et.al*, 2010). The value and need to further increase the production of wheat is recognized widely.

Plant breeding, in principle involves creation and exploitation of the genetic variation for different traits of economic importance. However, a century-long bread wheat breeding effort for developing high yielding varieties resulted in reduction of genetic diversity, thus

emphasizing the need for creation of variability in wheat breeding programs (Fu and Somers, 2009).

The winter wheat gene pool possesses huge diversity for various agronomic traits, can be exploited for widening the genetic base and improving the yield and other agro-morphological traits of spring wheat varieties. Winter wheat being hardier for most of the biotic and abiotic stress tolerance, presents themselves as a source for these traits. Also since there is no crossability barriers it becomes imperative to use these as donors for the improvement of spring wheat. One of the important approaches of wheat breeding is hybridization followed by selection. Choice of suitable parents is the basic need of any crop improvement program. Precise information on the nature and degree of genetic diversity helps plant breeders in selecting the parents for targeted hybridization (Samsuddin, 1985). Genetic divergence analysis estimates the extent of diversity existing among selected genotypes. The cluster analysis is an appropriate method for determining family relationships i.e. to determine the extent of genetic affinity or distance of genotypes from each other (Mellingers, 1972).

In order to benefit from transgressive segregation, genetic distance between parents is necessary (Joshi *et al*., 2004) that can be estimated by Euclidean distance (Hoque and Rahman, 2006). Principal component analysis (PCA) allows natural grouping of the genotypes and is precise indicator of differences among genotypes. The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only (Mohammadi, 2002).

Relationships among traits of economic importance impact the breeding strategies along with selection procedure. If all breeding objectives were positively correlated, selection would not be difficult than selecting for a single trait. If all breeding objectives were either positively correlated or independently inherited, selection would not be too difficult either (Mohammadi or Amri, 2011). Strong negative genetic correlations between breeding traits often exist, which make breeding very challenging (Yan and Wallace., 1995; Lewis, 2006). The genotype-by-trait (GT) biplot is a statistical tool for evaluating cultivars based on multiple traits and for identifying lines that are superior. Entries identified for agro-morphological and physiological traits hence could be candidates for use as parents in a breeding program (Yan and Rajcan, 2002). The GT biplot analysis allows visualization of genetic correlation among traits (Ma *et al*., 2004; Yan and Fregeau-Reid, 2008) and also helps in studying genotype by trait relationships.

The objectives of this study were to (i) estimate the diversity among tested genotypes for agronomic and physiological traits and (ii) to identify the genotype and trait relationship by GT biplot.

MATERIALS AND METHODS

Plant Material

The plant material comprised 11 advance lines bulked from winter x spring crosses and 12 lines selected from International Nurseries supplied from CIMMYT, Mexico (Table 1). These 23 wheat strains (WS) numbered WS1 to WS23 along with 2 checks HD2967 and GW322 were evaluated in a 5 X 5 simple lattice design with 2 replications during crop season of 2012-13 at the Directorate of Wheat Research Karnal farm ((Latitude 29^0 43’ N, longitude 76^0 58’ E and altitude 245 m). These genotypes were sown in plots of 6 rows of 5 meter length with a row spacing of 20 cm. A pre-emergence spray of Penidmethalin (Stomp) 30EC was applied at the rate of 1 kg AI/Hectare for control of weeds. The recommended dose of fertilizer (N:P:K:: 150:60::40 Kg/ha) for North Western Plains Zone of India was applied as: ½ dose of Nitrogen and full dose of phosphorus and potash as basal and remaining nitrogen was top dressed in 2 splits at the time of first and second irrigation. During the crop season 4 irrigations were applied at CRI (Crown Root Initiation), first node (45DAS), jointing (65 DAS) and milking (105 DAS) stages.

The observations were recorded on the following parameters:

Early vigour (EV): The early vigour was ssessed visually at the 6-7-leaf stage (1 to 5, with 5 being the best).

Table 1. Tested genotypes of wheat with their pedigree.

Wheat Strain #	Pedigree	Source
WS 1	UP 2572/WUGENG 8025	Winter X Spring derivatives
WS2	UP2425/SPARTANKA-KAK-HORI-DOLI//PHR 1010	Winter X Spring derivatives
WS3	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS4	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS5	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS6	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS7	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS8	90Zhong65/UP 2572	Winter X Spring derivatives
WS9	90Zhong65/UP 2572	Winter X Spring derivatives
WS10	UP2572/F35.70	Winter X Spring derivatives
WS11	HD 2590/Amigo//UP2572	Winter X Spring derivatives
WS12	SOKOLL/3/PASTOR//HXL7573/2*BAU	28th SWASN 3013 (2010-11)
WS13	KLDR/PEWIT1//MILAN/DUCULA	28th SAWSN 3111 (10-11)
WS14	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/KIRITATI	5TH STEMRRSN 6056 (2010-11)
WS15	SERI82/SHUHA'S'//PASTOR2	CWANA 11th SBWON 69 (2010-11)
WS16	IBWSN34/QAFZAH24//SUNBRI	CAWAN 11th SBWON 134 (2010-11)
WS17	Grackle	I CSISA HTEM 10218 (2009-10)
WS18	PAURAQUE	I CSISA HTEM 10236 (2009-10)
WS19	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	3CSISA DRYT 5221 (2010-11)
WS20	WAXWING*2/CIRCUS	ICSISA SB6737
WS21	GOUBARA-1/2*SOKOLL	19th SAWYT 340 (2011-12)
WS22	SOKOLL//FRTL/2*PIFED	19th SAWYT 331 (2011-12)
WS23	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	19th SAWYT 326 (2011-12)
HD 2967	ALD/CUC//URES/HD2160M/HD2278	Check
GW 322	PBW173/GW196	Check

Leaf firing (Fl): Yellowing of leaf tips at 65 days after sowing was recorded for presence (1) and absence (0).
Days to heading (DTH): It was calculated as days taken from sowing to emergence of 50% spikes in a plot.
Plant height (PtHT): Measured at time of maturity in centimeters from the ground level up to terminal spikelet, excluding awns.
Number of tillers/m (Tillers): Productive tillers were counted from 3rd and 4th row in one meter row length of the plot and averaged.
Chlorophyll content Index: The chlorophyll content Index (CCI) of flag leaf was measured from the middle part of the leaf by OPTI-SCIENCES CCM 200 plus chlorophyll meter at 3 stages:
CHL I: Chlorophyll Concentration Index at time of when the crop was fully flowered.
CHL II: Chlorophyll Concentration Index during grain filling duration (10 days after CHL I)
CHL III: Chlorophyll Concentration Index during at physiological maturity
Grain yield (Yld): The grain yield was recorded in grams from the gross plot area (6.0 m^2.)

Statistical analysis

The Euclidean distances were calculated by the Wards method and dendrogram was constructed to examine the relationships among different genotypes (Karon' ski and Calin' ski, 1973; Sokal and Rohlf, 2003). Principal component analysis, cluster analysis and Genotype by trait Biplot was constructed from Paleontological Statistics 3.01 Software Package **(**University of Oslo, Norway) (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Results obtained from the experiment are described and discussed under the following heads.

Principal Component Analysis

Principal component analysis (PCA) reveals the significance of the major contributor to the total variation at each axis of differentiation. The eigen values helps in determining the number of factors to be retained. Therefore, in this analysis the first factor retains the information contained in 3.788 of the original variables (Table 2).

Table 2. Vector loadings and percentage explained variation by the first 5 PCs.

Traits	PC1	PC2	PC3	PC4	PC5
EV	-0.2500	0.4443	0.3779	0.1523	-0.2156
FI	0.4352	-0.1897	0.3659	0.1026	0.0834
DTH	0.2388	0.1165	0.6752	-0.2649	0.3957
PtHT	-0.2654	0.05568	0.2527	0.7799	0.1998
Tillers	-0.3394	0.1638	-0.1495	-0.3156	0.7217
CHLI	0.3956	0.3349	-0.2617	0.1699	0.2008
CHLII	0.3535	0.3714	-0.3287	0.2995	0.2499
CHLIII	0.1452	0.6344	0.06791	-0.2597	-0.3555
Yield	-0.4505	0.2657	-0.0002889	-0.04693	0.0063
			Loadings		
Eigen value	3.7886	1.5585	1.2160	0.8766	0.7018
Individual Percentage	42.0960	17.3170	13.5110	9.7401	7.7978
Cumulative variance	42.0960	59.4130	72.9240	82.6641	90.4619

The first 5 principal components, PC1 to PC5 obtained from original data accounted for 90.5% of total variation. Among all principal components PC1 contributed maximum (42.09%) to the total variation.

Characters with largest absolute value closer to unity within the first principal component influences the clustering more than those with lower absolute value closer to zero (Chahal and Gosal, 2002). Accordingly, the major contributing traits for diversity in first principal component were leaf firing and grain yield. Presence of positive and negative correlation trends between the components and the variables are interpreted by positive and negative loading. Similarly, for second principal component (PC2) CHL III and early vigor were major contributors for the diversity.

The major contributing character for the diversity in the third principal component 3 (PC3) was days heading, while plant height in principal component four (PC4) and tillers per meter and days to heading in principal component five (PC5).

Customary, one variable is selected from these identified groups depending on respective loadings. Hence, for the first group grain yield is the best choice, which had the largest loading from PC1, CHL III for the PC2, days to heading for the third group and plant height and tillers/m for fourth and fifth group respectively.

Cluster Mean Analysis

The dendrogram was generated to examine the relationships among different genotypes based on Euclidean distances, calculated by the Wards method is presented in Figure 1, mean values of the traits in each cluster studied are presented in Table 3 and the feature of each cluster is described below.

Looking at the dendrogram, it could be seen that 2 major clusters were formed, Cluster I and cluster II. Cluster I have 2 sub-clusters which could be named as ‘a’ and ‘b’. Cluster ‘a’ consisted of 5 genotypes *viz.*, WS9, WS10, WS 11, WS 1 and check variety GW322. The genotypes in this cluster had shown no leaf firing and were early in flowering with dwarf height, moderate yield and high CCI at maturity. Similarly cluster ‘b’ comprised of 5 winter x spring derivatives (WS3, WS4, WS 5, WS6 and WS7) derived from common cross (UP2425/Centruk//PHR1010) which were characterized by high leaf firing, delayed flowering, tall plant height, low tillering and low yield.

Table 3. Mean value of the traits in the 4 clusters**.**

Cluster		EV	FI	DTH	PtHT	Tillers	CHLI	CHLII	CHLIII	Yield
Cluster I a	Mean	3.80	0.00	95.40	90.49	115.90	24.91	23.75	3.91	2444.10
	Max	4.00	0.00	105.50	93.55	138.00	27.60	25.45	8.00	2562.50
	Min	3.00	0.00	90.50	85.40	93.50	22.55	22.15	1.95	2279.50
Cluster I b	Mean	3.80	1.00	106.70	90.55	85.20	27.14	23.80	3.25	1760.60
	Max	4.00	1.00	111.00	90.90	100.00	31.55	25.85	5.10	1980.50
	Min	3.50	1.00	103.00	90.10	78.00	24.60	21.80	1.90	1558.00
Cluster II c	Mean	4.21	0.00	99.79	92.86	128.07	21.15	18.44	2.73	2970.64
	Max	5.00	0.00	104.00	94.10	176.00	26.15	24.90	6.60	3110.50
	Min	3.00	0.00	96.00	91.75	102.50	14.20	11.65	1.60	2849.00
Cluster II d	Mean	4.56	0.00	100.00	93.79	129.69	22.46	19.54	3.09	3294.81
	Max	5.00	0.00	105.00	102.50	167.50	26.35	23.65	6.70	3459.50
	Min	3.50	0.00	95.50	90.10	105.00	17.55	14.55	1.55	3171.50

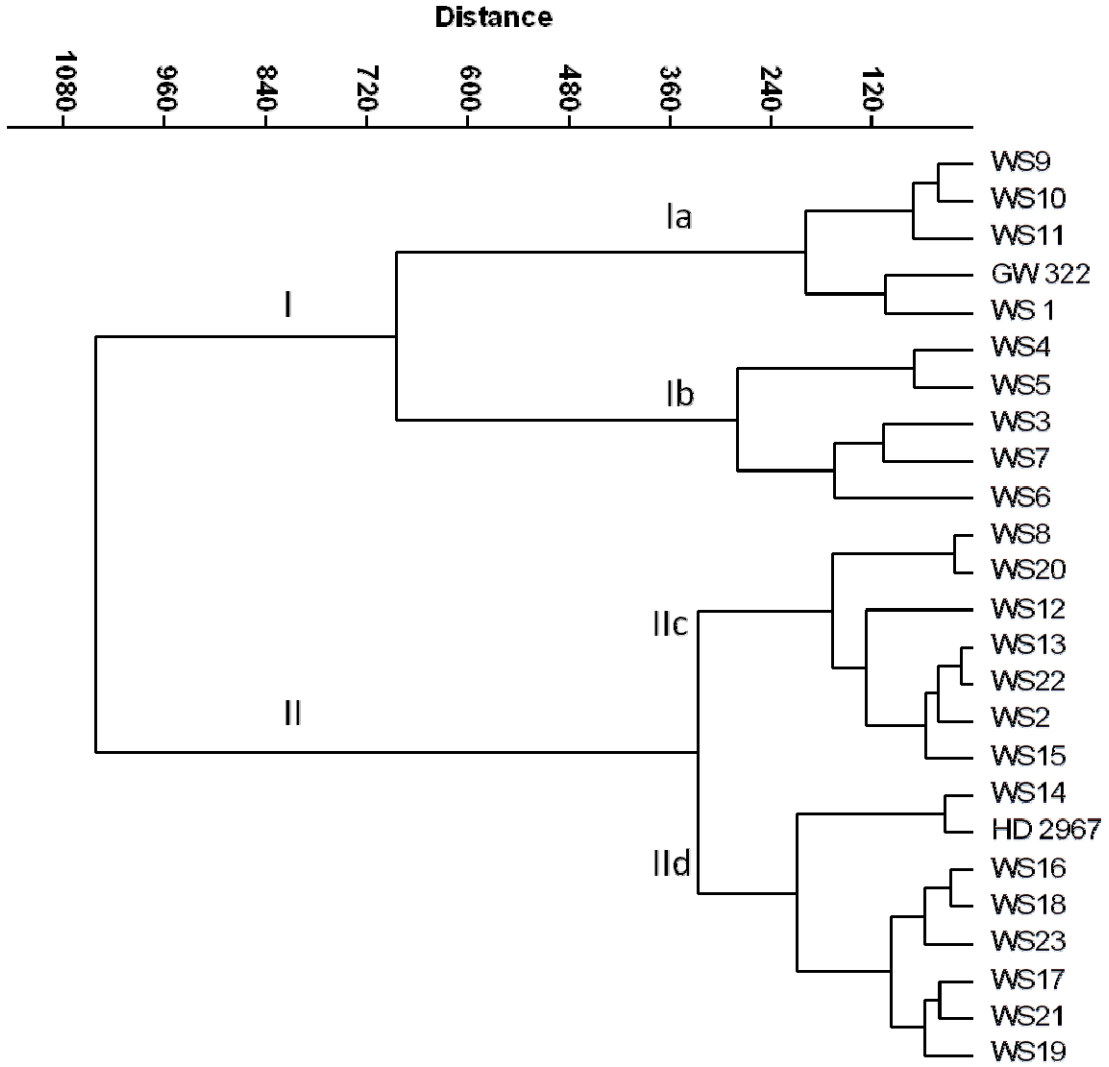

Figure 1. Dendrogram depicting genetic relationships among 25 wheat genotypes.

These genotypes possess high CHL I but high chlorophyll was not converted to high yield due to shorter time for grain filling caused by terminal heat. Both cluster represented 40% of total genotypes with an equal representation of 20% for each cluster.

Cluster II have 2 sub-clusters 'c' and 'd'. Cluster 'c' comprised of 7 genotypes (WS8, WS20, WS12, WS13, WS22, WS2 and WS 15) with characteristic feature of moderate early vigor, no leaf firing, moderate tillering and average yield. This cluster represented 28% of total genotypes. Cluster 'd' consisted of 8 genotypes *viz.*,WS 14, WS16, WS18, WS23, WS17, WS21 and WS19) and the recently released variety HD2967 with characteristic feature of high early vigor, no leaf firing, medium plant height, high tillering and high grain yield. This was the largest cluster with a representation of 32% of total genotypes.

The cluster analysis showed that there was significant genetic variability among wheat genotypes tested that indicated the presence of

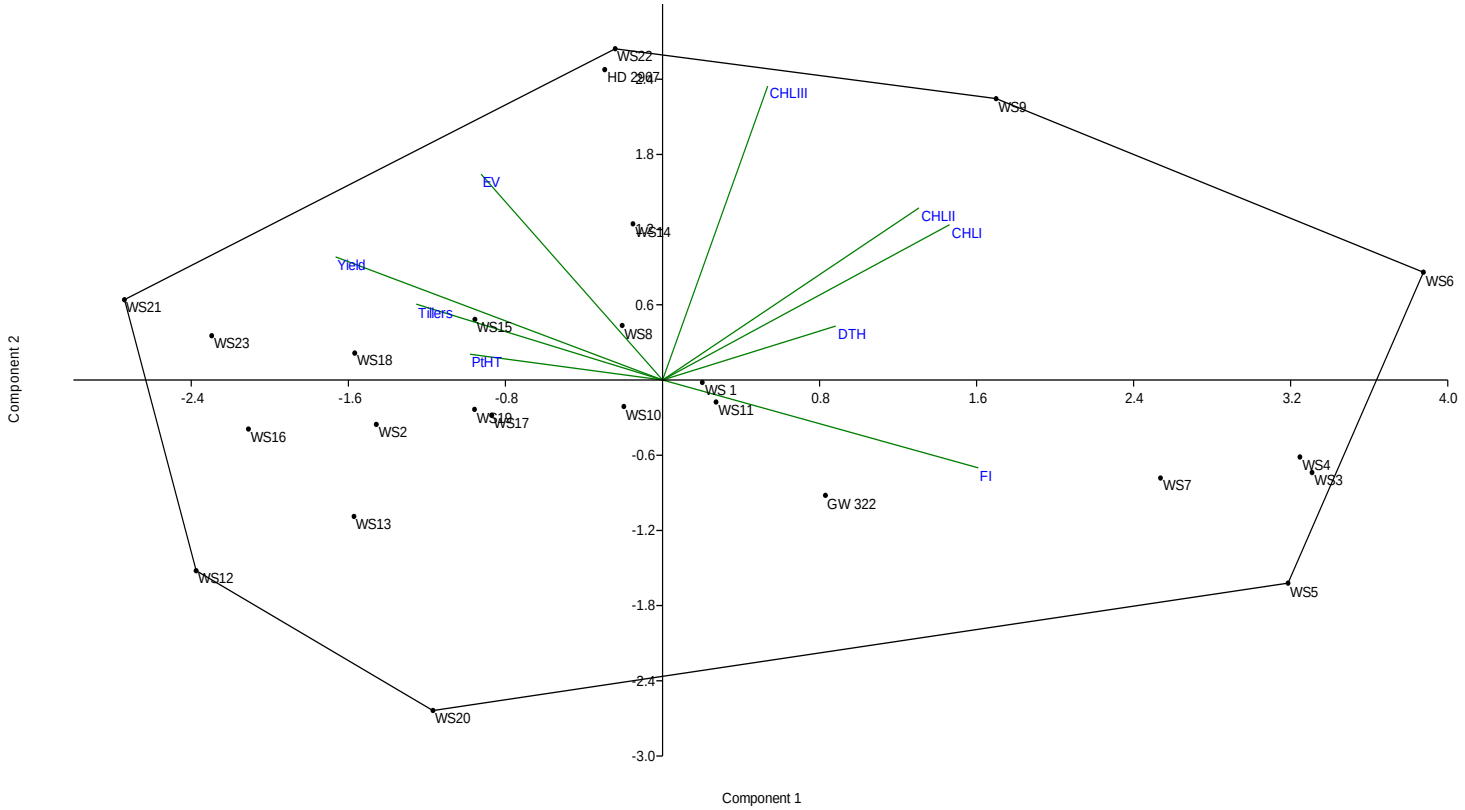

Figure 2. Genotype by trait (GT) biplot comprising of 25 wheat genotypes studied for 9 traits in 2012-13 cropping season.

excellent opportunity to bring about improvement through hybridizing genotypes from different clusters. The winter x spring derivatives from cluster II should be crossed with the genotypes of cluster I to reduce the flowering time. High yielding genotypes from cluster IV could be further tested for their combining ability. Thus the genotypes present in different clusters can be hybridized to assemble desirable traits with higher heterotic potential.

Genotype by trait (GT) biplot

The biplot (Figure 2) displays the relationship of 25 wheat lines for 9 traits. The GT biplot of the mean performance of the wheat genotypes explained the 59.4 % of the total variation of the standardized data.

In GT biplot, a vector drawn from origin to each trait facilitates the visualization of interrelationships among traits. The vector length of the trait measures the magnitude of its effects on the yield (Yan and Tinker, 2005). The polygon view of GT biplot is best to visualize the interaction pattern between genotypes and traits, provided the biplot should explain a sufficient amount of the total variation. The correlation coefficient between any 2 traits is approximated by the cosine of the angle between their vectors (Yan and Rajcan, 2002). On this premise, 2 traits are positively correlated if the angle between their vectors is an acute angle (< 90°) while they are negatively correlated if their vectors are an obtuse angle (> 90°) (Yan and Kang, 2003).

Across the 25 tested wheat genotypes grain yield was positively associated with tillers/m and early vigor and negatively associated with leaf firing. Although the plant height was positively associated with grain yield but its magnitude was less. However, in regular breeding programs taller genotypes are not preferred. Days to heading were negatively associated with grain yield this might be due to the shortened grain filling duration. CCI measured at 3 stages were positively associated with each other indicating the loss of chlorophyll was similar in most of the genotypes tested. The vector trait yield made a 180 degree angle with leaf firing indicating traits to be opposite in genotype ranking. The distance between genotype and the biplot origin is a unique measure of the genotype (i.e., how it differs from an “average” genotype), which is a hypothetical genotype that has an average level for all traits and is represented by the biplot origin (Yan and Fregeau-Reid, 2008). Therefore, genotypes WS 5, WS6, WS9, WS22, WS21, WS12 and WS 20 with long vectors are those that have extreme values for one or more traits. Such genotypes may or may not be superior, but they may be useful as parents for some useful traits

CONCLUSION

The cluster analysis showed that there is significant genetic variability among tested wheat genotypes that indicates the presence of excellent opportunity to bring about improvement through hybridizing genotypes from different clusters. The winter spring derivatives need further improvement regarding days to heading. The GT biplot showed that the grain yield was positively associated with tillers/ m and early vigor and negatively associated with leaf firing.

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RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (1) 49-59, 2015

BREEDING POTENTIAL OF INDETERMINATE TOMATO (*Solanum lycopersicum* L.) ACCESSIONS USING D^2 ANALYSIS

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SUMMARY

The exploration of genetic diversity is a pre-requisite in any breeding program for effective selection of superior accessions. Hence, a study was conducted on 19 accessions of tomato collected from Indian Institute of Vegetable Research, Varanasi and Vegetable Research Station, Junagadh Agricultural University, Junagadh, India, to assess the value and magnitude of genetic divergence using Mahalanobis D^2 statistics. Wide genetic diversity was observed among the accessions which were grouped into five clusters by Tocher's method based on D^2 values. The clusters III and IV contained highest number of accessions (6) followed by clusters I and II both had 3 accessions and cluster V had one accession. The clustering pattern indicated that there was no association between geographical distribution of accessions and genetic divergence. The diversity among the clusters was measured by inter-cluster distance. The maximum inter-cluster D^2 value was observed between the cluster I and IV (11347.2) followed by cluster I and III (10921.8). Therefore, selection of divergent parents based on these cluster distance would be useful in selecting accessions for hybridization and formulating a comprehensive strategy to develop superior hybrids or superior segregants in tomato. The information so generated can be effectively utilized for improving the specific traits in future breeding programs of tomato. Cluster mean analysis indicated cluster I showed maximum performance for fruit yield per plant (3270.6 g), cluster II recorded minimum days to flowering (57.3 days) and cluster V showed low mean for leaf curl incidence percentage (20.0). The accessions in cluster I, II and V could serve as direct source for development of high yielding varieties, early flowering and resistance to leaf curl disease, respectively.

Keywords: Breeding potential, genetic divergence, multivariate analysis, tomato, yield

Short statement summary: Information on the extent of genetic diversity among accessions is very important in crosses between groups with maximum genetic divergence that would be more responsive for improvement since they are likely to produce desirable recombination and segregation in their progenies after hybridization. To have this type of knowledge, research on genetic diversity is very essential.

Manuscript received: September 19, 2014; Decision on manuscript: December 6, 2014; Manuscript accepted: January 24, 2015

Communicating Editor: Bertrand Collard

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of family Solanaceae is one of the most important vegetable crops in India as well as around the world (Cheema and Dhaliwal, 2005; Kumar *et al.,* 2010; Kumar and Dudi, 2011; Osekita and Ademiluyi, 2014). It has a chromosome number of 2n = 24 (Rick, 1969). Tomato is native of West Coast of South America (Mexico and Peru) and was cultivated by Indians about 500 B.C. long before arrival of Spaniards (Rehman *et al.,* 2000; Tasisa *et al*., 2012). Tomato is the most important vegetable crop next only to potato because of its wider adaptability, high yielding potential and multipurpose uses (Reddy *et al*., 2013b). In India, tomato occupies an area of 0.87 million ha with a production of 17.50 million ton and productivity of 20.11 tons per hectare (FAO, 2012). Tomato is grown as annual or short lived perennial herbaceous plants. It has a taproot, and the growth habit of the plant is determinate, semi-determinate and indeterminate. It finds a very important role in every kitchen with enormous role in food and nutritional security. It also has a very important and significant position in the post-harvest industry (Kumar *et al*., 2010). It is an important protective food because of its special nutritive value as it contains abundant and well balanced nutrition consisting of minerals, vitamins, dietary fiber, citric acid etc. (Thapa *et al*., 2014). Ascorbic acid may play a key role in delaying the pathogenesis of a variety of degenerative diseases, such as cardiovascular disease, certain cancers, cataracts and it also prevents DNA mutation induced by oxidative stress (Byers and Guerrero, 1995; Marchioli *et al*., 2001; Lutsenko *et al*., 2002). Lycopene and β-carotene are the tomato carotenes which present the highest nutritional value (Tomlekova *et al*., 2007; Glogovac *et al*., 2010). Lycopene may alleviate chronic diseases such as cancer and coronary heart disease (Canene-Adams *et al*., 2005; Omoni and Aluko, 2005; Kun *et al*., 2006).

Looking at its commercial importance, there is utmost need to develop newer varieties/accessions/hybrids with higher yield, disease resistance, and processing traits. For this purpose the breeders choose genetically distant parents, genetic diversity plays an important role in breeding vegetables, because hybrids derived from the lines of diverse origin display more heterosis than those between closely related strains (Lahbib *et al*., 2012; Srivastava *et al*., 2014). The greater is parental diversity, the greater is the chance of developing higher yielding breeding lines (Joshi and Dhawan, 1966; Singh *et al*., 2012). Estimation of genetic divergence also allows breeders to eliminate some parents in downsizing the scale of hybridization activities and concentrate their efforts in a smaller number of combinations (Fuzzato *et al*., 2002). Although tomato is a self-pollinated crop, there is genetic diversity not only in the morphological features but also in the quality attributes as reported by Abushita *et al*., 1997. Among the various methods identified/developed to study the genetic divergence in the genotypes/accessions, the Mahalanobis D^2 (Mahalanobis, 1936) is reliable and most frequently used. For the first time use of this technique for assessing the genetic variability in plants was suggested by Rao (1952). It is a very useful technique of measuring genetic divergence (Meena and Bahadur, 2013; Sharma and Devi, 2013; Ramanjaneyulu *et al*., 2014; Srivastava *et al*., 2014). Further, grouping of the accessions based on Tocher's method will be more useful in choosing suitable parents for heterosis breeding (Prashanth *et al*., 2008). D^2 analysis is a useful tool in quantifying the degree of divergence between biological population at genotypic level and to assess relative contribution of different components to the total divergence, both at the inter- and intra-cluster levels (Singh and Singh, 1980; Singh *et al*., 2006a; Ara *et al*., 2009). Genetic diversity analysis also reveals the redundancy of accessions with respect to a particular trait or combination of traits, which avoids wastage of resources. The progenies derived from diverse parents are expected to show a broad spectrum of genetic variability and provide better scope to isolate superior recombinants. Therefore, genetically diverse genotypes/ accessions should be used in a hybridization program to get superior recombinants. Keeping these points in mind, this study was undertaken to access and evaluate the genetic diversity in indeterminate tomato

accessions collected from diverse origin on the basis of yield and quality traits and to identify superior accessions for future use.

MATERIALS AND METHODS

Experimental location

A field study was carried out during the season 2012-13 at Vegetable Research Farm, Department of Horticulture, SHIATS, Allahabad, India. The city is situated in south-eastern part of the state Uttar Pradesh, India (25° 28' N latitude and 81° 54' E longitude) and at a mean altitude of 98 m above sea level. Geologically, the area forms a part of the Indo-Gangetic alluvial plains.

Climate and soil of experimental field

The climate of Allahabad is characterized as humid sub-tropical with an average annual rainfall of 1027 mm (40.4 inches). The rainfall is monsoonal in nature with around 75% received during July-September. The soil of the experimental field was loamy sand in texture, low in available nitrogen and organic matter, comparatively rich in available phosphorus and medium in available potassium with slightly alkaline reaction. The mean monthly agro-meteorological observations were recorded during the crop season (Figure 1).

Experimental material

The experimental materials comprised of 19 indigenous accessions of indeterminate tomato collected from Indian Institute of Vegetable Research (IIVR), Varanasi and Vegetable Research Station (VRS), JAU, Junagadh, India. For raising good and healthy seedlings, the seeds were treated with carbendazim using 2.0 g per kg of seed. After that the seeds of 19 accessions of tomato were sown in the nursery bed on 30 September, 2012 and their seedlings were transplanted on 4th November, 2012 in small plots (2.0 m × 2.0 m) in open-field where row-to-row and plant-to-plant spacing was 60 cm x 60 cm that contained 16 plants. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications. All the recommended agronomic package of practices were followed (like staking, earthing up, irrigation, weeding etc.), as recommended for commercial tomato production.

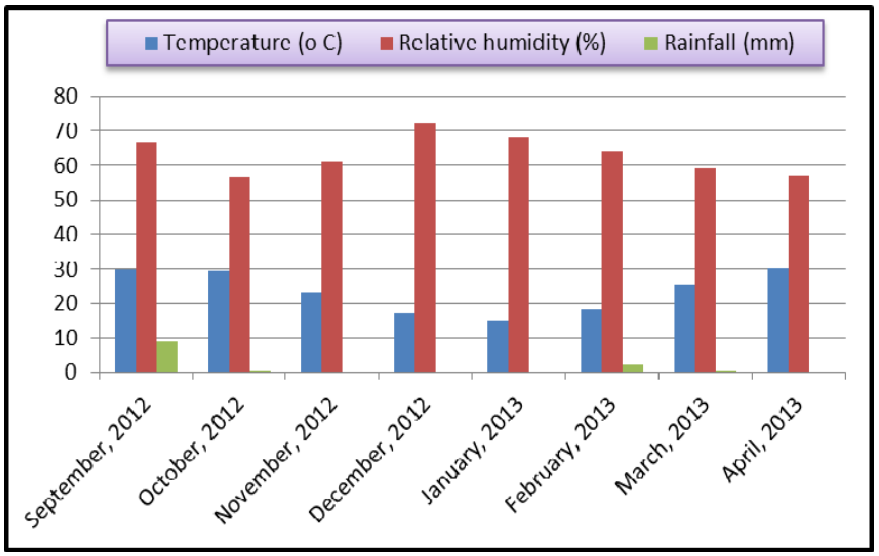

Figure 1. Mean monthly agro-meteorological observations recorded during crop season in 2012-13.

Experimental data

The observation were recorded on 5 randomly selected plants per replication for each accession on 15 quantitative characters i.e., [1] plant height (cm), [2] number of branches per plant, [3] number of leaves per plant, [4] days to flowering, [5] number of flower clusters per plant, [6] number of flowers per plant, [7] number of fruits per plant, [8] fruit set per cent, [9] fruit weight (g), [10] radial diameter of fruit (mm), [11] polar diameter of fruit (mm), [12] fruit yield per plant (g), [13] leaf curl incidence percentage (based on the scale given by Joshi and Choudhary, 1981), [14] TSS °Brix (by using a hand refractometer, Model: ATAGO, Tokyo, Japan) and [15] ascorbic acid (mg/100g) was estimated using 2,6-dichlorophenol indophenol method as illustrated by AOAC (1975).

Statistical analysis

Mahalanobis D^2 analysis

The data collected were subjected to multivariate analysis utilizing Mahalanobis D^2 statistic as suggested by Mahalanobis (1936) and Rao (1952) using statistical software WINDOSTAT 9.1 developed by INDOSTAT services Ltd. Hyderabad, India. Accessions were grouped into various clusters following Tocher's method as suggested by Rao (1952).

RESULTS AND DISCUSSION

On the basis of D^2 values, the 19 accessions were grouped into five highly divergent clusters (Table 1 and Figure 2), indicating adequate genetic diversity for selecting superior and diverse parents which can be exploited for any breeding program. The cluster divergence was proved by the high inter-cluster and low intra-cluster D^2 values. The perusal of data (Table 1) depicted that clusters III and IV each had the maximum number of accessions (6) followed by cluster I and cluster II with 3 accessions in each cluster and cluster V is solitary consisting of only one accessions. The clustering pattern in the present study showed that accessions of different geographical areas were clubbed in one group indicating that there was no parallelism between genetic diversity and geographical origin. These results are similar to the findings of Peter and Rai (1976); Martin *et al.* (1981); Rai *et al*. (1998); Dharmatti *et al*. (2001); Mohanty and Prusti (2001); Parthasarathy and Aswath (2002); Joshi and Kohli (2003); Singh *et al*. (2006a); Mehta and Asati (2008); Singh *et al*. (2006b); Singh *et al*. (2008); Basavaraj *et al*.(2010); Kumar *et al*. (2010); Shashikanth *et al*. (2010); Kumar *et al*. (2013); Meena and Bahadur (2013). On the other hand, the accessions that originated in one region had been distributed into different clusters, indicating that accessions with same geographic origin could have under gone change for different characters under selection. This could be due to selection or genetic drift, which helps in creating more diversity rather than genetic distance. Therefore, selection of accessions for hybridization to generate diverse new gene combinations should be based on genetic diversity rather than geographic diversity. This finding is in conformity with the findings of Ganesh *et al*. (2007); Pawar *et al*. (2013).

Table 1. Clustering pattern of 19 accessions of indeterminate tomato based on D^2 statistics.

Cluster Number	Number of Accessions	Accessions Included
I	3	2012/TOINDVAR-2, 2012/TOINDVAR-3, 2012/TOINDVAR-4
II	3	EC 620430, EC 620432, EC 620434
III	6	2011/TOINDVAR-4, 2011/TOINDVAR-5, 2012/TOINDVAR-1, EC 620421, GT-1,
IV	6	2011/TOINDVAR-1, 2011/TOINDVAR-2, 2011/TOINDVAR-3, EC 620437, EC
V	1	ANGOORLATA

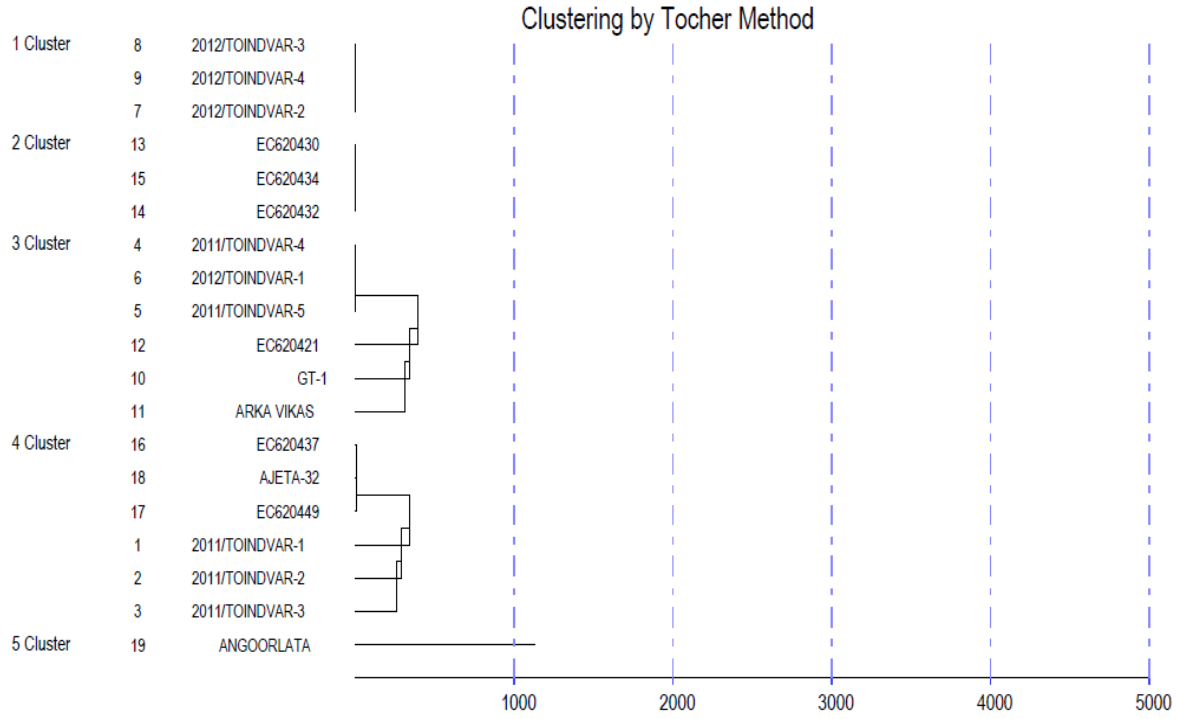

Figure 2. Dendrogram showing clustering patterns of 19 indeterminate tomato accessions grown under open field conditions (Tocher's method).

In line with this, Yashavantakumar *et al*. (2009) grouped 70 tomato genotypes into 7 clusters. Similarly, Chernet *et al*. (2014) clustered 36 genotypes into 6 distinct clusters using Mahalanobis distance; Iqbal *et al*. (2014) grouped 47 tomato genotypes into 5 clusters; Ghosh *et al*. (2009) clustered 40 genotypes into 6 clusters; Gonçalves *et al*. (2009) grouped 40 tomato accessions into 5 diverse clusters. Meena and Bahadur (2013) also employed Mahalanobis distance (D^2) to classify 30 tomato germplasm in to 6 clusters.

The divergence within the cluster (intra-cluster distance) indicates the divergence among the accessions falling in the same cluster. On the other hand, inter cluster divergence suggests the distance (divergence) between the accession of different clusters. The intra and inter cluster D^2 values among 19 accessions presented in Table 2 and Figure 3 revealed that cluster V showed minimum intra-cluster D^2 value (0) followed by cluster II (7.0), whereas, maximum intra-cluster D^2 value (913.9) was shown by cluster III followed by cluster IV (779.3) and cluster I (9.9), revealing considerable genetic divergence among the accessions of this cluster and was due to both natural and artificial selection forces among the accessions (Rathi *et al*., 2011). Minimum inter-cluster D^2 value was observed between the cluster III and IV (1870.3) followed by cluster II and IV (3097.5) indicated close relationship among the accessions included in these clusters. Maximum inter-cluster D^2 value was observed between the cluster I and IV (11347.2) followed by cluster I and III (10921.8), cluster I and II (6949.5), cluster II and V (6930.8), cluster IV and V (6757.2) and cluster I and V (4879.6) indicated that the accessions belonging to these groups were genetically most diverse and the accessions included in these clusters can be used as a parent

Table 2. Intra (Diagonal) and Inter-cluster distance (D^2) among indeterminate tomato accessions.

Clusters	I	II	III	IV	V
I	9.94	6949.50	10921.84	11347.25	4879.60
II		7.07	4095.74	3097.54	6930.82
III			913.93	1870.39	4185.23
IV				779.37	6757.26
V					0.00

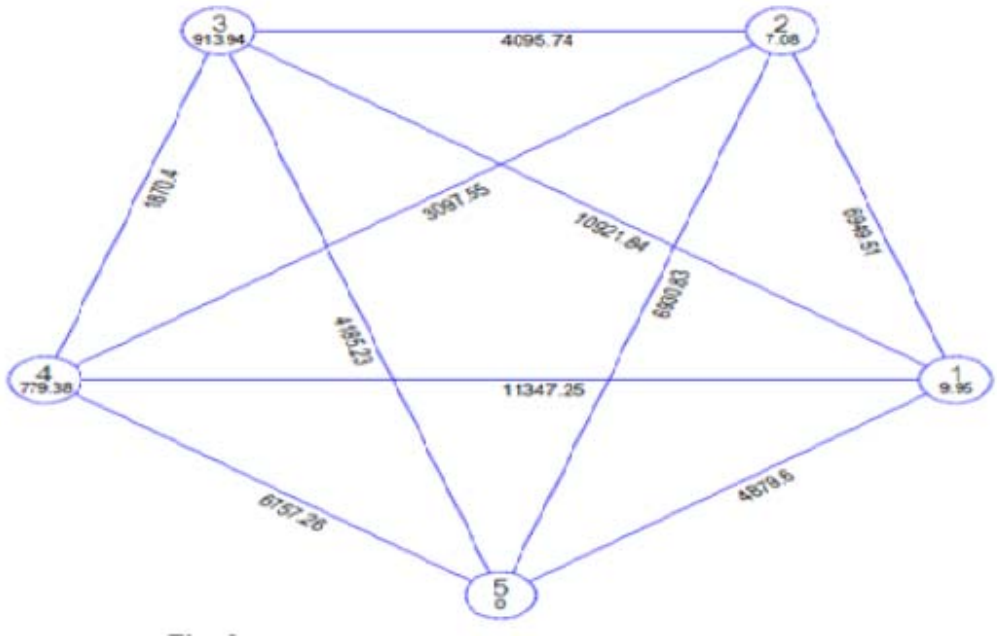

Figure 3. Mahalnobis Euclidean Distance (not to scale)

in hybridization program to get higher heterotic hybrids from the segregating population. Similar results were revealed by Babu and Patil (2004); Mehta *et al.* (2004); Mehta and Asati (2008); Meena and Bahadur (2013).

Several authors also reported diversity in the accessions of tomato by assessing genetic divergence on the basis of quantitative traits following Mahalanobis D^2 statistics (Guirgis *et al.*, 1994; Basavaraj *et al.*, 2010; Evgenidis *et al.*, 2011; Meena and Bahadur, 2013; Reddy *et al.*, 2013a). Average inter and intra-cluster distances revealed that, in general, inter-cluster distances were much higher than those of intra-cluster distances, suggesting homogeneous and heterogeneous nature of the accessions lines within and between the clusters, respectively. These results are in accordance with the findings of Mahesha *et al.* (2006); Sekhar *et al.* (2008); Meena and Bahadur (2013) in tomato. The percentage contribution of 15 characters for genetic divergence (Table 3) showed that leaf

Table 3. Percentage contribution of different characters to genetic divergence among indeterminate tomato accessions.

S. No.	Source	Time Ranked 1st	Contribution (%)
1.	Plant Height cm (at 120 DAT)	3	1.7
2.	Branches/ Plant (at 120 DAT)	0	0.0
3.	Leaves/ Plant (at 120 DAT)	2	1.2
4.	Days to flowering (50%)	0	0.0
5.	Flower Clusters/ Plant	2	1.2
6.	Flowers/ plant	27	15.8
7.	Fruits/ plant	0	0.0
8.	Fruit set (%)	1	0.6
9.	Fruit weight (g)	11	6.4
10.	Radial diameter of Fruit (mm)	3	1.7
11.	Polar diameter of Fruit (mm)	43	25.1
12.	Fruit yield/ plant (g)	1	0.6
13.	Leaf curl Incidence %	66	38.6
14.	TSS (°Brix)	3	1.7
15.	Ascorbic acid (mg/ 100 g)	9	5.3

DAT: Days after transplanting; mm: millimeter; g: gram.

curl incidence percentage contributed maximum (38.6%) towards genetic divergence followed by polar diameter of fruit (25.1%), number of flowers per plant (15.8%) and fruit weight (6.4%). Mohanty and Prusti (2001); Reddy *et al*. (2013a); Singh *et al*. (2008) also observed such maximum contribution for fruit weight to total divergence of tomato accessions. De *et al*. (1988) opined that traits contributing maximum towards the D^2 values needed to be given more emphasis for deciding the clusters to be taken for the purpose of choice of parents for hybridization.

Further, for crop improvement, intercrossing among accessions with outstanding mean performance was suggested by Roy and Sharma (1996); Kumar *et al*. (2013), and the reliable conformity for this can be known on the basis of cluster means. The cluster means of accessions (Table 4) revealed considerable genetic differences between the groups. Accessions in cluster I showed maximum performance for fruit yield per plant (3270.6 g) which indicates that the accessions included in this cluster could effectively be used for the crop improvement program for increasing yield. Cluster II recorded minimum days to flowering (57.3 days), whereas, maximum mean value for radial diameter of fruit (59.8 mm) and polar diameter of fruit (50.2 mm). It reveals that if a breeding program is aimed at earliness, then accessions in cluster II can be selected (Meena and Bahadur, 2013). Cluster III showed high mean performance for TSS (4.9 °Brix) and ascorbic acid (43.9 mg/100g). Cluster IV recorded maximum performance for fruit weight (56.9 g) and leaf curl incidence percentage (24.5). Cluster V showed high mean value for plant height (171.7 cm), number of branches per plant (21.1), number of leaves per plant (221.2), days to flowering (63.0 days), number of flower clusters per plant (27.2), number of flowers per plant (136.0), number of fruits per plant (76.2) and fruit set percentage (56.0), whereas, low mean performance for leaf curl incidence percentage (20.0). Depending upon the breeding objective, the potential lines to be selected from different clusters as parents in a hybridization program may be based on genetic distance. In accordance to the findings, Edang *et al*. (1971); Hazra *et al.* (2010) reported that the clustering pattern could be utilized in choosing parents for cross combinations likely to generate the highest possible variability for various economic characters.

Table 4. Cluster mean of 19 indeterminate tomato accessions for 15 traits.

Character / Clusters	Plant height at 120DAT	Branches / plant at 120DAT	Leaves/ plant at 120 DAT	Days to flowering	Flower clusters/ plant	Flowers/ plant	Fruits/ plant	Fruit set %	Fruit weight (g)	Radial diameter of fruit (mm)	Polar diameter of fruit (mm)	Fruit yield/ plant (g)	Leaf curl incidence (%)	TSS °Brix	Ascorbic acid (mg/ 100g)
I	131.7	17.1	194.7	59.8	19.5	111.2	57.9	52.1	56.0	57.6	48.7	3270.6	24.4	4.2	33.6
II	158.8	19.6	204.1	57.33	22.33	126.5	56.7	45.4	48.8	59.8	50.2	2774.7	22.7	4.5	38.8
III	145.8	18.0	203.6	57.34	22.31	130.6	53.6	41.3	54.7	52.2	44.9	2920.7	22.5	4.9	43.9
IV	143.6	18.2	190.8	58.9	22.2	124.7	55.5	44.7	56.9	54.0	49.9	3119.3	24.5	4.3	33.7
V	171.7	21.1	221.2	63.0	27.2	136.0	76.2	56.0	34.6	34.7	41.2	2637.6	20.0	4.8	42.3

DAT: Days after transplanting; mm: millimeter; g: gram.

CONCLUSIONS

For generating wide spectrum of variability intercrossing of accessions of cluster I for fruit yield per plant; cluster II for minimum days to flowering, radial diameter of fruit and polar diameter of fruit; cluster III for TSS and ascorbic acid; cluster IV for fruit weight; and cluster V for plant height, number of branches per plant, number of leaves per plant, number of flower clusters per plant, number of flowers per plant, number of fruits per plant, fruit set percentage and low leaf curl incidence percentage. The accessions of the cluster I for highest mean yield per plant and cluster V for low leaf curl incidence percentage can be utilized as donor parents in hybridization program for enhancing the yield and minimum leaf curl incidence of other accessions grouped in a cluster and can be fixed by selecting transgressive segregants followed by continued selection in advanced generations which may lead to development of high yielding varieties with desired component characters. Accessions from highly divergent clusters may also be utilized in a breeding program for development of high yielding varieties with desirable attribute and can also be utilized in heterosis breeding program for development of F_1 hybrids with superior yield and quality characters. Hybridization between divergent parents is likely to produce wide variability and transgressive segregation with high heterotic effects. The above findings indicated that the smallest inter-cluster distance was observed between cluster III and IV (1870.3) followed by cluster II and IV (3097.5). The lines belonging to these clusters were relatively closer to each other, in comparison to lines grouped in other clusters. This analysis would be useful to avoid selecting parents from genetically homogeneous clusters, and maintain a relatively broad genetic base for breeding.

ACKNOWLEDGEMENTS

The authors are highly grateful to IIVR, Varanasi and VRS, JAU, Junagadh, India for providing accessions of tomato.

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RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (1) 60-69, 2015

COMBINING ABILITY AND GENE ACTION STUDIES FOR YIELD AND QUALITY TRAITS IN BABY CORN (*Zea mays* L.)

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SUMMARY

An investigation was carried out to assess the combining ability and nature of gene action in baby corn genotypes using a diallel mating design (without reciprocal crosses) using 7 homozygous lines namely, FDM 10, FDM 8, FDM 7, FDM 12, FDM 37, FDM 14 and FDM 36. The experiment was set up in a randomized complete block design (RCBD) with 2 replications during *rabi* season of 2010-11. General combining ability studies revealed that FDM 14 was the best combiner for major yield contributing characters including days to tasseling, plant height, number of leaves per plant, number of baby corns per plant, baby corn length, baby corn weight and baby corn yield per plot and FDM 37 was best combiner for yield and quality traits including number of baby corns per plant, baby corn yield per plot, total sugars and reducing sugars. However, the estimates of specific combining ability showed the desirable SCA effects in crosses FDM 37 x FDM 14 and FDM 7 x FDM 14 for all traits studied except for days to tasseling and non-reducing sugars. Gene action analysis revealed preponderance of both additive and non-additive genes for yield and its contributing characters.

Keywords: Genotype x environment interaction, plant breeding, quantitative trait loci, mapping

Short summary statement: In the present investigation, yield and quality attributes of baby corn was governed by both additive and non-additive genes. When the character is under the control of additive gene action, simple selection may be followed to their improvement. If non additive genes are predominant for the traits, heterosis breeding may be effective for their improvement.

Manuscript received: October 11, 2014; Decision on manuscript: December 9, 2014; Manuscript accepted: January 14, 2015.

Communicating Editor: Bertrand Collard

INTRODUCTION

Maize is unique among the cereals on account of its amenability to diverse uses and it has huge potential in the present era of crop diversification. India is emerging as one of the potential baby corn producing countries due to low cost of production and high demand within the country. Baby corn is a young finger like unfertilized cob of maize harvested early within 1-3 days of silk emergence. Baby corn is a good option for crop diversification and it suits to peri-urban agriculture. Further, there is a great potential to earn foreign exchange through export of fresh/canned baby corn and its processed products. Another important feature of baby corn is safe vegetable to eat as it is almost free from residual effects of pesticides as the young cob is rapped with husk and well protected from insect and diseases. Despite manifold uses of baby corn, very little information on breeding strategies followed for

improvement in baby corn (Chauhan and Mohan, 2010).

It is a fact that selection of parents on the basis of their mean performance does not necessarily lead to desired results (Rai and Asati, 2011). Therefore, devising a sound breeding strategy to improve the yield of this crop is of paramount importance. Combining ability analysis help breeders in choosing suitable genotypes as parents for hybridization and superior cross combinations through GCA and SCA studies, respectively (Rodrigues and da Silva, 2002; Rai and Asati, 2011). At the same time, it also elucidates the nature and magnitude of different types of gene action involved, which is essential for an effective breeding program. Hence, this investigation was undertaken to study the estimates of general and specific combining ability and gene action in baby corn for yield components and quality characters.

MATERIALS AND METHODS

Experimental material

The present experiment was carried out at Department of Forage Crops, Tamil Nadu Agricultural University, Coimbatore, by involving 7 genetically diverse baby corn inbreds viz., FDM 10, FDM 8, FDM 7, FDM 12, FDM 37, FDM 14 and FDM 36 were used as parents (Table 1) and crossed in diallel mating design following Model-I, Method-II of Griffing (1956). This method of combining analysis includes one way crosses and their parents. This method is used when reciprocal differences are not significant. This is most commonly used method of combining ability analysis from a diallel cross (Singh and Narayanan, 1993)

Experimental methods

The parents and their resulting 21 F_1s were raised in a randomized complete block design (RCBD) with 2 replications during *rabi* season of 2010-11. Each plot consisted of 2 rows of 5 m length and spacing between rows and plants adopted were 30 and 20 cm respectively (3 m^2). One plant per hill was maintained and recommended package of practices was followed to raise a healthy crop (Crop production guide, 2013). Observations on baby corn yield and its component and quality traits including days to 50% tasseling (DFT), plant height (PHT), number of leaves per plant (NOL), number of baby corns per plant (NBC), baby corn length (BCL), baby corn weight (BCW), baby corn yield per plot (BCY), total sugars (TSR), reducing sugars (RSR) and non-reducing sugars (NRS) were recorded on 5 randomly selected plants from each plot.

Mean data was subjected for analysis of general combining ability (GCA), specific combining ability (SCA) and gene action as per method given by Griffing (1956) (method 2 and model I) using the software WINDOSTAT (version 7.1).

Table 1. List of parent material used with study and its origin.

Parents	Origin
FDM 7	Directorate of maize research-winter nursery centre, Hyderabad
FDM 8	Directorate of maize research-winter nursery centre, Hyderabad
FDM 10	Directorate of maize research-winter nursery centre, Hyderabad
FDM 12	Directorate of maize research-winter nursery centre, Hyderabad
FDM 14	Department of forage crops, Coimbatore.
FDM 36	Maize research station, Vagarai
FDM 37	Maize breeding station, Coimbatore

RESULTS AND DISCUSSION

Analysis of variance for GCA and SCA presented in Table 2 revealed that mean sum of squares of combining ability for various yield and yield contributing and quality characters were highly significant for all the characters except baby corn weight, for which SCA effect was found non-significant, showing that the behavior of the best hybrids can be foreseen by using inbreds with high general combining ability. The lack of specific combining ability indicates low genetic complementation among inbreds for alleles which show dominance (Cruz and Regazzi, 1997). This could be explained by assuming that the tested inbreds have the same origin and are obtained from the self-pollination of one indigenous composite-cross of baby corn.

The mean squares of SCA were larger than those of GCA in all the characters except for reducing sugars indicating the preponderance of non-additive gene action in the control of most of the characters. Involvement of non-additive gene action for the characters in present investigation is also in consonance with the findings of Anantha (2004) and Selvarani (2007) for days to tasseling, Geetha and Jayaraman (2000), Anantha (2004) and Prakash and Ganguli (2004) for plant height, Jayakumar and Sundaram (2007) for number of leaves per plant, Rodrigues and da Silva (2002) for baby corn length and Suneetha *et al*. (2000) for non-reducing sugars. On other hand, higher GCA value recorded for reducing sugars than its SCA value indicating involvement of additive genes. This suggested that simple selection would be effective to make desirable improvement of the character under study.

Estimates of general combining ability for various traits have been presented in Table 3. The estimates of GCA effects exhibited that the parent FDM 14 was the best general combiners for most studied characters *i.e.* days to tasseling, plant height, number of leaves per plant, baby corn length, baby corn weight and baby corn yield per plot and parent FDM 37 exhibited desirable GCA effects for plant height, number of baby corns per plant, baby corn yield per plot, total sugars and reducing sugars. So, these parents could be used extensively in hybrid breeding program to increase baby corn yield with quality. Similar to the present investigation were also reported for the following characters *i.e.* plant height (Vacaro *et al.*, 2002; Malik *et al*., 2004 and Shalim Uddin *et al*., 2006), number of leaves per plant (Mahajan and Khehra, 1991 and Reddy and Agarwal, 1992), number of baby corns per plant, baby corn length, baby corn weight and baby corn yield per plot (Rodrigues and da Silva, 2002), total sugars and reducing sugars (Selvarani, 2007).

High positive estimates of specific combining ability in absolute values indicates that hybrid performance is relatively superior or inferior to parent lines general combining ability, showing the importance of non-additive interactions resulting from the complementation degree among parent lines in relation to frequency of alleles in loci with some dominance, while low estimates of specific combining ability in absolute value indicates that hybrids behave as expected in relation to general combining ability of parent lines (Vencovsky and Barriga, 1992). In the selection of parent lines used to produce hybrids, the effect of a specific combining ability analyzed in an isolated way has a limiting value. Thus, other parameters should be considered such as the average of hybrids and general combining ability of the respective parent lines (Oliveira *et al*., 1998). Therefore, superior hybrid combinations, which are important for breeding, are involved with at least one parental line which has the most favorable effects of general combining ability (Cruz and Regazzi, 1997). Thus, it is possible to analyze the 2 hybrids that showed high performance for most of the yield and quality traits, such as FDM 37 x FDM 14 and FDM 7 x FDM 14 (Table 4).

Baby corn yield per plot in the FDM 37 x FDM 14 is associated with high effects of general combining ability of both the parent lines. Therefore, in this case, the high productivity is not due to dominant genetic effects of inbreds but to additive effects. In the FDM 7 x FDM 14 hybrid it is associated with the high effect of the general combining ability of the FDM 14 inbred with one of the highest effects of the estimated specific combining ability, since FDM 7 inbred showed lower general combining ability. In this case, the participation of a specific combining ability is

Table 2. Analysis of variance for combining ability analysis for baby corn yield and quality traits.

Sources	DFT	PHT	NOL	NBC	BCL	BCW	BCY	TSR	RSR	NRS
GCA	11.730**	4196.179**	3.404**	0.235**	4.745**	9.186**	447.7143**	1.020**	1.624**	0.226**
SCA	7.765**	1260.862**	1.135**	0.222**	1.365**	2.784	473.442**	0.167**	0.171**	0.111**
Error	2.055	283.329	0.651	0.033	0.871	2.914	18.277	0.007	0.002	0.011
σ^2 GCA	1.189	450.502	0.342	0.024	0.479	0.859	48.738	0.113	0.180	0.025
σ^2 SCA	6.738	1119.197	0.809	0.204	0.930	1.327	464.304	0.164	0.169	0.105
σ^2 GCA / σ^2 SCA	0.176	0.402	0.423	0.118	0.515	0.647	0.105	0.691	1.065	0.234

*and ** indicates significance at 5% and 1% level respectively.

Table 3. Estimates of mean and general combining ability effects (GCA) of the parents for baby corn yield and quality traits.

Parents	DFT		PHT		NOL		NBC		BCL	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
FDM 10	71.00	0.520	90.00	-19.400**	11.34	-0.630**	3.00*	0.180**	7.97	-0.281
FDM 8	72.00	0.470	99.17	-12.090**	11.84	-0.320	2.20	-0.14**	9.22	0.240
FDM 7	70.50	-0.530	111.84	-0.310	12.84	0.350	2.10	-0.050	7.60	-0.738 *
FDM 12	72.50	-0.140	69.84	-14.620**	11.84	-0.210	1.30	-0.250**	8.00	0.140
FDM 37	70.50	-1.860**	127.50	9.810*	10.34	-0.480*	3.20**	0.160**	7.84	-0.810*
FDM 14	76.00**	1.850**	232.34**	43.740**	14.84**	1.160**	2.00	0.100*	11.50**	1.340*
FDM 36	71.50	-0.310	94.00	-7.130	12.67	0.130	3.10*	0.010	8.52	0.100
Mean	72.00		117.81		12.24		2.41		8.66	
SE_d	1.43		16.83		0.80		0.19		0.93	
CD at 5%	2.94		34.50		1.65		0.39		1.91	
$SE_{(gi)}$		0.310		3.670		0.180		0.041		0.201

(Continued)

Parents	BCW		BCY		TSR		RSR		NRS	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
FDM 10	6.89	-0.560	132.00**	6.630**	3.21	-0.319*	2.80	-0.219*	0.42	-0.101 *
FDM 8	6.64	-0.280	105.00	-6.750**	3.81	-0 018	3.72**	0.157*	0.09	-0.173*
FDM 7	6.50	-0.937*	96.00	-3.700***	3.90**	0.054*	3.40**	0.035*	0.50	0.020
FDM 12	8.14	0.893*	62.00	-9.480**	3.03	-0.341 *	2.31	-0.647*	0.72*	0.305*
FDM 37	6.22	-0.530	148.00**	8.960**	4.24**	0.468*	3.75**	0.587*	0.49	-0.121 *
FDM 14	11.22*	1.885 *	88.00	4.960**	2.72	-0.259*	2.43	-0.305*	0.29	0.046
FDM 36	6.84	-0.460	138.00**	-0.630	4.41**	0.416*	3.67**	0.392*	0.75*	0.024
Mean	7.49		109.86		3.62		3.15		0.47	
SE_d	1.70		4.27		0.08		0.05		0.10	
CD at 5%	3.49		8.76		0.17		0.10		0.21	
$SE_{(gi)}$		0.371		0.933		0.018		0.010		0.023

Table 4. Estimates of mean and specific combining ability effects (SCA) of the hybrids for baby corn yield and quality traits.

Hybrids	DFT		PHT		NOL		NBC		BCL	
	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
FDM 10 x FDM 8	68.50	-1.520**	138.17	1.890	13.16	0.510*	2.10	-0.300**	7.40	-1.710**
FDM 10 x FDM 7	68.50	-0.520	166.17	18.110**	12.84	-0.490*	2.800*	0.320**	7.80	-0.330
FDM 10 x FDM 12	69.50	0.080	150.17	16.430**	13.34	0.560*	3.100**	0.820**	10.78	1.778**
FDM 10 x FDM 37	66.50	-1.190**	176.67	18.480**	13.50	1.010**	2.16	-0.530**	9.50	1.443**
FDM 10 x FDM 14	71.50*	0.080	193.17	1.050	14.67	0.530*	2.33	-0.310**	10.43	0.220
FDM 10 x FDM 36	70.50	1.250**	163.17	21.930**	13.00	-0.100	2.00	-0.550**	8.80	-0.170
FDM 8 x FDM 7	70.50	1.520**	166.67	11.300*	13.84	0.190	1.90	-0.260**	8.53	-0.120
FDM 8 x FDM 12	70.00	0.630	172.83	31.780**	13.84	0.750**	2.20	0.240**	10.97	1.441**
FDM 8 x FDM 37	68.50	0.860*	180.17	14.670**	13.00	0.190	2.16	-0.210**	7.28	-1.294**
FDM 8 x FDM 14	68.00	-3.360**	207.67	8.240	14.50	0.040	2.900**	0.590**	11.14	0.410
FDM 8 x FDM 36	67.00	-2.190**	169.50	20.950**	14.00	0.580*	1.90	-0.330**	11.60*	2.108**
FDM 7 x FDM 12	65.50	-2.860**	177.83	25.000**	13.67	-0.080	2.16	0.120*	9.15	0.602*
FDM 7 x FDM 37	64.00	-2.630**	195.67	18.390**	14.66	1.190**	2.20	-0.260**	5.97	-1.632**
FDM 7 x FDM 14	69.00	-1.360**	224.50*	13.300*	15.83*	0.710**	2.900**	0.500**	11.34*	1.588**
FDM 7 x FDM 36	69.00	0.800	184.83	24.510**	15.50	1.420**	2.20	-0.120*	8.53	0.030
FDM 12 x FDM 37	62.50	-4.520**	188.33	25.370**	13.84	0.920**	2.00	-0.260**	8.53	0.060
FDM 12 x FDM 14	69.50	-1.250**	208.67	11.780*	15.34	0.770**	2.20	0.00	9.30	-1.325**
FDM 12 x FDM 36	69.00	0.410	173.00	26.980**	13.34	-0.190	2.30	0.180**	9.68	0.300
FDM 37 x FDM 14	70.50	1.470**	236.17**	14.840**	14.66	0.380	3.200**	0.590**	10.25	0.575*
FDM 37 x FDM 36	62.50	-4.360**	198.50	28.040**	14.16	0.920**	2.17	-0.360**	8.66	0.230
FDM 14 x FDM 36	68.50	-2.080**	201.00	-3.380	14.66	-0.230	2.20	-0.270**	9.77	-0.818**
Mean	68.05		184.42		14.06		2.34		9.31	
SE_d	1.43		16.83		0.80		0.19		0.93	
CD at 5%	2.94		34.50		1.65		0.39		1.91	
$SE(_{sii})$		0.910		10.680		0.510		0.06		0.590

*and ** indicates significance at 5% and 1% level respectively

(Continued)

Hybrids	BCW		BCY		TSR		RSR		NRS	
	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
FDM 10 x FDM 8	4.20	-3.063**	99.00	-9.050**	2.82	-0.556**	2.65	-0.561**	0.17	0.000
FDM 10 x FDM 7	5.40	-1.206*	117.000*	5.910**	3.02	-0.428**	2.84	-0.253**	0.19	-0.169**
FDM 10 x FDM 12	10.30	1.863**	141.000**	35.680**	2.94	-0.113**	2.37	-0.037*	0.58	-0.069*
FDM 10 x FDM 37	8.99	1.974**	98.00	-25.760**	4.89**	1.033**	4.68**	1.039**	0.22	0.001
FDM 10 x FDM 14	8.97	-0.460	122.000**	2.240	3.22	0.085**	2.93	0.181**	0.28	-0.101**
FDM 10 x FDM 36	8.16	1.085*	84.01	-30.160**	3.52	-0.290**	3.16	-0.290**	0.36	0.000
FDM 8 x FDM 7	6.68	-0.200	81.00	-16.720**	3.81	0.060*	3.65**	0.185**	0.16	-0.127**
FDM 8 x FDM 12	11.88*	3.160**	101.00	9.060**	3.20	-0.154**	2.63	-0.148**	0.56	-0.010
FDM 8 x FDM 37	6.72	-0.580	100.00	-10.380**	4.26**	0.097**	3.94**	-0.077**	0.32	0.173**
FDM 8 x FDM 14	11.09	1.373**	129.000**	22.620**	3.53	0.099**	3.28	0.151**	0.26	-0.050
FDM 8 x FDM 36	8.50	1.137*	84.60	-16.180**	4.31**	0.199**	4.01**	0.184**	0.31	0.020
FDM 7 x FDM 12	7.97	-0.100	103.00	8.020**	3.76	0.334**	2.28	-0.376**	1.46**	0.699**
FDM 7 x FDM 37	4.85	-1.787**	110.00	-3.430**	3.80	-0.430**	3.74**	-0.150**	0.05	-0.285**
FDM 7 x FDM 14	11.97*	2.916**	126.000**	16.570**	3.62	0.107**	3.36	0.352**	0.26	-0.242**
FDM 7 x FDM 36	6.55	-0.160	103.00	-0.830	4.39**	0.212**	3.82**	0.126**	0.57	0.085**
FDM 12 x FDM 37	8.66	0.200	91.00	-16.650**	3.32	-0.515**	2.95	-0.258**	0.37	-0.255**
FDM 12 x FDM 14	9.36	-1.520**	105.00	1.350	3.74	0.627**	2.06	-0.261**	1.68**	0.888**
FDM 12 x FDM 36	8.45	-0.090	115.00	16.950**	3.61	-0.178**	3.43*	0.418**	0.18	-0.595**
FDM 37 x FDM 14	10.53	1.071*	153.000**	30.910**	4.10**	0.173**	3.82**	0.270**	0.27	-0.091**
FDM 37 x FDM 36	7.89	0.770	98.00	-18.490**	5.06**	0.458**	4.82**	0.569**	0.22	-0.119**
FDM 14 x FDM 36	7.47	-2.063**	99.00	-13.490**	3.74	-0.130**	3.13	-0.225**	0. 61	0.094**
Mean	8.31		107.60		3.75		3.31		0.42	
SE_d	1.70		4.28		0.08		0.05		0.10	
CD at 5%	3.49		8.76		0.17		0.10		0.21	
$SE_{(sii)}$		1.080		1.230		0.050		0.030		0.070

*and ** indicates significance at 5% and 1% level respectively

Table 5. Components of genetic variation in a 7 x 7 half-diallel set of baby corn.

Genetic components	DFT	PHT	NOL	NBC	BCL	BCW	BCY	TSR	RSR	NRS
D	2.6131	2722.6880**	1.6922**	0.4710**	1.4194*	1.3038	944.6581**	0.4057**	0.3927**	0.0488
F	-1.1480	1392.5610*	0.4364	0.7855**	-0.5493	-3.2766	1667.6410*	0.0210	-0.2683	0.0074
H_1	23.1845**	2957.4640**	2.4353**	1.0388**	4.3952**	6.4256*	2374.9110**	0.7108*	0.7788*	0.4811
H_2	20.9215**	2664.3960**	2.3381**	0.6490*	4.1912**	6.9855**	1468.1140*	0.6063*	0.5800*	0.3886
h^2	45.3915**	12965.9200**	9.6284**	0.0039	1.0072	1.1260	10.6500	0.0490	0.0745	0.0002
E	1.0536	149.8326	0.3153	0.0271	0.4323	1.7730	8.8181	0.0032	0.0011	0.0052
$(H_1/D)^{1/2}$	2.9787	1.0422	1.1996	1.4851	1.7597	2.2200	1.5856	1.3237	1.4083	3.1400
$H_2/4H_1$	0.2256	0.2252	0.2400	0.1562	0.2384	0.2718	0.1545	0.2133	0.1862	0.2019
KD/KR	0.8626	1.6503	1.2408	3.5607	0.8019	0.2771	3.5115	1.0400	0.6095	1.0496
h^2/H_2	2.1696	4.8664	4.1180	0.0060	0.2403	0.1612	0.0073	0.0808	0.1284	0.0004

*and ** indicates significance at 5% and 1% level respectively

significant for hybrid yield, contributing almost to the general combining ability from both inbreds, regarding the dominance and epistasis effects (Gardner, 1963). However, the hybrid FDM 10 x FDM 12 exhibited high specific combining ability for number of baby corns per plant and baby corn length and FDM 10 x FDM 37 showed highest specific combining ability for total sugars and reducing sugars. About 6 hybrids exhibited higher mean performance and SCA effects for baby corn yield per plot. In respect of baby corn superiority, it is decided by its quality. Hence, the crosses FDM 37 x FDM 14 and FDM 7 x FDM 14 recorded the high mean and SCA effects for yield and quality traits.

The estimates of D, H_1, H_2, F and E parameters along with its components obtained from diallel analysis (Hayman, 1954; Rai and Asati, 2011) are presented in Table 5. Significant value of additive component (D) and non-additive component (H_1 and H_2) observed for plant height, number of leaves per plant, number of baby corns per plant, baby corn length, baby corn yield per plot, total sugars and reducing sugars indicated involvement of both additive and non-additive gene action for the expression of these characters. However, non-additive effects (dominance component) were significantly higher than its additive component (D) for number of leaves per plant, number of baby corns per plant, baby corn weight, baby corn yield per plot, total sugars and reducing sugars. This suggests the preponderance of non-additive (dominance) genetic variation in the expression of these characters. Earlier, preponderance of non-additive gene action in baby corn has also been reported by Rodrigues and da Silva (2002) for baby corn length which is in agreement with our findings.

The mean degree of dominance $(H_1/D)^{1/2}$ was more than unity for all the characters studied which indicated presence of over dominance for expression of these characters. The ratio of $H_2/4H_1$, which was less than 0.25 in all the characters, indicated asymmetrical distribution of positive and negative genes in the parents. The value of KD/KR was higher than the unity for all the characters except days to 50% tasseling, baby corn length, baby corn weight and reducing sugars indicating presence of greater proportion of dominant gene in the expression of these traits. Whereas, for days to tasseling, baby corn length, baby corn weight and reducing sugars KD/KR value recorded less than unity, indicating presence of greater proportion of recessive genes.

In this present study, most of the yield and quality traits includes plant height, number of leaves per plant, number of baby corns per plant, baby corn length, baby corn yield per plot, total sugars and reducing sugars is governed by both additive and non-additive genes but later is predominant suggesting that bidirectional recurrent selection could be adopted for the improvement of these traits, whereas additive gene action was found prominent for the expression of the characters *i.e.* days to tasseling, baby corn weight and non-reducing sugars indicating simple selection could be effective for bringing improvement of this traits in baby corn.

CONCLUSION

FDM 14 and FDM 37 were the best among the 7 parents as it showed desirable mean and GCA effects for most of yield and its contributing traits and yield and quality traits respectively. Therefore these parents could be used extensively in hybrid breeding program with a view to increase baby corn yield with quality. Furthermore, based on mean and SCA effects 2 hybrids FDM 37 x FDM 14 and FDM 7 x FDM 14 were proved to be the best to increase the baby corn yield with better quality. For varietal improvement, these crosses could also be utilized for exploiting promising recombinants and it could be useful towards enhancing baby corn yield and quality.

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RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (1) 70-78, 2015

CORRELATION ANALYSIS OF TRAITS IN ELITE GENOTYPES OF CORIANDER

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SUMMARY

Coriander (*Coriandrum sativum* L.) has been cultivated for a long time in different parts of India and the world. This study aimed to identify the role of coriander seeds towards improved nutrition, which is essential for biological processes. The present investigation was carried on 64 coriander genotypes to identify the traits associated with seed yield and their attributes. Results revealed that seed yield plant^{-1} exhibited a positive and significant correlation with number of fruits umbel^{-1} but negative correlation with days to 50% flowering and days to 80% maturity. Almost all genotypes studied revealed diverse properties, making them suitable genetic materials for breeding homogenous coriander cultivars. Our research goal was to elucidate the diversity of agronomic, physiological and yield traits in coriander. In the present investigation, genotypic correlation coefficients were higher than the phenotypic ones because of the masking effect of genotypes for the expression of characters. Seed yield plant^{-1} exhibited a positive and significant correlation with number of fruits umbel^{-1} but was negatively correlated with days to 50% flowering and 80% maturity, whereas number of fruits umbellet^{-1} expressed a positive significant correlation with number of fruits umbel^{-1} and 1000-seed weight. A positive correlation was also noted between 1000-seed weight and number of fruits umbel^{-1}.

Keywords: Coriander, correlation coefficient, genotypic correlation, phenotypic correlation

Manuscript received: February 4, 2014; Decision on manuscript: May 23, 2014; Manuscript accepted: July 5, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an annual herb that belongs to the family Apiaceae (Umbelliferae) and is known to originate from the Mediterranean region (Hedberg and Hedberg, 2003). The Egyptians call this herb the "spice of happiness," probably because of its aphrodisiac property (Uhl 2000). It shows broad adaptation as a crop around the world, growing well under different kinds of soil and weather conditions (Simon 1990, Pedro *et al.*, 2008), even at extreme latitudes and elevations. In addition, the short life cycle of most coriander cultivars allows farmers to fit their cultivation into some part of the growing season in almost any region. Coriander has long been cultivated in the Mediterranean region, southern Europe, Asia Minor and the Caucasus. In recent years, principal commercial coriander producers include members of the former Soviet Union, Hungary, Poland, Romania, Czech Republic, Slovakia, Morocco, Canada, India, Pakistan, Iran, Turkey, Guatemala, Mexico and Argentina (Kiehn and Reimer 1992, Agri-facts 1998).

In India, coriander is mainly cultivated in Andhra Pradesh, Madhya Pradesh, Tamil Nadu, Assam, Maharashtra, Rajasthan, Uttar Pradesh and Punjab. More recent applications include its use as a green vegetable and as flavoring of dishes and food such as pickles, sauces and confectionery. It is increasingly becoming important in the oleo-chemical industry. Coriander produces a lot of nectar and attracts many different insects for pollination. It is also a good melliferous (yielding or producing honey) plant, allowing bees to collect a lot of honey. All parts of the plant are edible but it is mostly grown for the green vegetable and seeds, which when dried, have a mild aromatic flavor. Thus, it is mainly grown as a dual-purpose crop for seeds (dry fruits) as well as for its leaves.

There are many different uses of coriander and these are based on the different parts of the plant. Traditional uses of the plant, which are based on the primary products (the fruits and the green herb), are two-fold: medicinal and culinary. During industrialization, specific chemical compounds of coriander were recognized and identified, and these became important raw materials for industrial use and further processing. The seeds are used as an important ingredient in various food preparations and the leaves are often used for garnishing dishes. The leaves, stalks and seeds of coriander contain certain essential oils. The essential and fatty oils of the fruits are used in industry, either separately or combined. Coriander freely cross-pollinates and does so without showing signs of inbreeding depression. Although coriander is one of the most important spices, very little attention has been given for its improvement. There are few recognized commercial varieties in India. It is necessary to develop more suitable varieties for seed production to fulfill the increasing demand for this spice crop.

A germplasm collection with good variability for desirable characters is a basic requirement of any crop improvement program (Singhania *et al*., 2006). Knowledge of the magnitude and direction of interrelationship between yield and its component characters is of great importance in breeding programs to select desirable types. The present study addresses this goal by examining 64 species of coriander.

MATERIALS AND METHODS

The experiment was carried out at the Vegetable Research Farm, Department of Horticulture, College of Agriculture, JNKVV, Jabalpur (MP) during the rabi seasons of 2010-11 and 2011-12. Sixty-four diverse genotypes were raised in Randomized block design with 3 replications. The seeds were split into 2 mericarps and sown directly into experimental plots with a spacing of 30 x 10 cm between 2 consecutive rows and plant.

Chlorophyll content in coriander leaves was estimated by the use of a chlorophyll meter. Correlation coefficients were calculated for all quantitative character combinations at the phenotypic, genotypic and environmental levels using the formula given by Miller *et al*. (1958). The genotypic, phenotypic and environmental correlations were computed by substituting the corresponding variance and covariance in the above mentioned formula. The estimation of covariance between 2 traits was derived in the same way as that for corresponding variance components.

RESULTS

Correlation provides the degree and direction of a relationship between variables at phenotypic, genotypic and environmental levels. Correlation coefficients were calculated at these three levels for all possible combinations of 14 characters (Tables 1, 2 and 3). Results indicated that genotypic correlation coefficients in general were of higher magnitude than the corresponding phenotypic correlation coefficients. Phenotypic correlation generally gives an idea about the association between 2 variables. The results of the present study are discussed below.

With regard to the results of first year (Table 1), plant height at maturity recorded a positive and significant association with chlorophyll content at 60 DAS (0.392), seed yield plant^{-1} (0.260), days to 80% maturity (0.152), days to 50% flowering (0.148) and number of primary branches plant^{-1}(0.137).

Table 1. Estimates of genotypic and phenotypic correlation coefficients for yield and its contributing characters in coriander (1st year).

Character		Chlorophyll content at 60 DAS	No. of primary branches	No. of fruiting nodes	Days to 50% flowering	Days to 80% maturity	No. of umbels plant^{-1}	No. of umbellets umbel^{-1}	No. of fruits umbellet^{-1}	No. of fruits umbel^{-1}	Diameter of fruit (mm)	Vegetative yield (kg)	1000-seed weight (g)	Seed yield plant^{-1} (g)
Plant height at maturity	G	0.405	0.155	0.061	0.150	0.151	0.092	0.211	0.069	0.167	-0.052	0.123	0.043	0.262
	P	0.392**	0.137*	0.060	0.148*	0.152*	0.080	0.111	0.060	0.086	-0.035	0.114	0.043	0.260**
Chlorophyll content at 60 DAS	G		0.419	0.129	0.275	0.251	0.194	0.291	0.211	0.208	-0.016	0.243	0.071	0.361
	P		0.360**	0.126	0.266**	0.240**	0.176*	0.124	0.187**	0.104	0.009	0.240**	0.070	0.335**
No. of primary branches	G			0.519	-0.008	-0.030	0.528	0.719	0.521	0.711	0.417	-0.13	-0.034	0.600
	P			0.465**	0.004	-0.007	0.441**	0.359**	0.390**	0.348**	0.279**	-0.103	-0.032	0.522**
No. of fruiting nodes	G				0.121	0.122	0.961	0.907	0.268	0.784	0.021	-0.046	-0.016	0.846
	P				0.124	0.126	0.920**	0.476**	0.233**	0.476***	0.022	-0.038	-0.018	0.819**
Days to 50% flowering	G					0.991	0.128	0.183	-0.163	-0.052	-0.053	0.307	-0.287	0.315
	P					0.976**	0.121	0.116	-0.145*	-0.039	-0.040	0.283**	-0.282**	0.303**
Days to 80% maturity	G						0.128	0.213	-0.174	-0.031	-0.081	0.282	-0.273	0.326
	P						0.109	0.146*	-0.147*	-0.019	-0.063	0.267**	-0.270**	0.310**
No. of umbels plant^{-1}	G							0.861	0.190	0.676	0.066	-0.034	-0.083	0.869
	P							0.431**	0.147*	0.445**	0.038	-0.037	-0.083	0.819**
No. of umbellets umbel^{-1}	G								0.593	1.043	0.174	0.090	0.146	0.896
	P								0.289**	0.464**	0.035	0.031	0.07	0.428*
No. of fruits umbellet^{-1}	G									0.831	-0.027	-0.097	0.289	0.175
	P									0.480**	-0.003	-0.062	0.257**	0.158*
No. of fruits umbel^{-1}	G										-0.133	-0.001	0.190	0.745
	P										-0.033	-0.008	0.110	0.400**
Diameter of fruit (mm)	G											-0.272	-0.126	0.135
	P											-0.199**	-0.092	0.100
Vegetative yield (kg)	G												-0.123	0.130
	P												-0.119	0.119
1000-seed weight (g)	G													-0.106
	P													-0.104

*, **Significant at 5 and 1% level, respectively.

Chlorophyll content at 60 DAS showed a positive and highly significant correlation with number of primary branches plant^{-1} (0.360), seed yield plant^{-1} (0.335), days to 50% flowering (0.266), days to 80% maturity (0.240), vegetative yield plot^{-1} (0.240), number of fruits umbellet^{-1} (0.187) and number of umbels plant^{-1} (0.176). Highly significant and positive association of primary branches plant^{-1} was observed with seed yield plant^{-1} (0.522), number of fruiting nodes plant^{-1} (0.465), number of umbels plant^{-1} (0.441), number of fruits umbellet^{-1} (0.390), number of umbellets umbel^{-1} (0.359), number of fruits umbel^{-1} (0.348) and diameter of fruits (0.279).

The correlation of number of fruiting nodes plant^{-1} was significant and positive with number of umbels plant^{-1} (0.920), seed yield plant^{-1} (0.819), number of umbellets umbel^{-1} (0.476), number of fruits umbel^{-1} (0.476) and number of fruits umbellet^{-1} (0.233). Days to 50% flowering had highly significant and positive association with days to 80% maturity (0.976), seed yield plant^{-1} (0.303) and vegetative yield plot^{-1} (0.283). The association was negative and significant with 1000-seed weight (-0.282) and number of fruits umbellet^{-1} (-0.145). Days to 80% maturity had a significant and positive correlation with seed yield plant^{-1} (0.310), vegetative yield plot^{-1} (0.267), and number of umbellets umbel^{-1} (0.146), whereas 1000-seed weight (-0.270) and number of fruits umbellet^{-1} (-0.147) showed significant and negative association.

Highly significant and positive association of number of umbels plant^{-1} was recorded with seed yield plant^{-1} (0.819), number of fruits umbel^{-1} (0.445), number of umbellets umbel^{-1} (0.431) and number of fruits umbellet^{-1} (0.147). A very strong positive and significant association of number of umbellets umbel^{-1} with number of fruits umbel^{-1} (0.464), seed yield plant^{-1} (0.428) and number of fruits umbellet^{-1} (0.289) was observed. The association of number of fruits umbellet^{-1} was significant and positive with number of fruits umbel^{-1} (0.480), 1000-seed weight (0.257) and seed yield plant^{-1} (0.158). Number of fruits umbel^{-1} expressed a positive and significant correlation with seed yield plant^{-1} (0.400) while fruit diameter exhibited a negative and significant correlation with vegetative yield plot^{-1} (-0.199).

In the second year (Table 2), plant height at maturity had a significant and positive association with chlorophyll content at 60 DAS (0.414), seed yield plant^{-1} (0.238), days to 50% flowering (0.165) and days to 80% maturity (0.158). The association of chlorophyll content at 60 DAS was significant and positive with seed yield plant^{-1} (0.344), number of primary branches plant^{-1} (0.333), days to 50% flowering (0.287) and 80% maturity (0.252), vegetative yield plot^{-1} (0.230), number of umbels plant^{-1} (0.182) and number of fruits umbel^{-1} (0.149). Number of primary branches plant^{-1} had significant and positive association with seed yield plant^{-1} (0.573), number of umbels plant^{-1} (0.517), number of fruiting nodes plant^{-1} (0.468), number of umbellets umbel^{-1} (0.403) and number of fruits umbellet^{-1} (0.395), fruit diameter (0.287) and number of fruits umbel^{-1} (0.264).

A strong positive association of number of fruiting nodes plant^{-1} was observed with number of umbels plant^{-1} (0.951), seed yield plant^{-1} (0.805), number of umbellets umbel^{-1} (0.609), number of fruits umbel^{-1} (0.409) and number of fruits umbellet^{-1} (0.316). Days to 50% flowering showed positive and highly significant correlation with days to 80% maturity (0.988), vegetative yield plot^{-1} (0.306) and seed yield plant^{-1} (0.285). On the other hand, it was negatively associated with 1000-seed weight (-0.272). Days to 80% maturity had a positive association with vegetative yield plot^{-1} (0.306) and seed yield plot^{-1} (0.283), however; it exhibited a significant negative association with 1000-seed weight (-0.268) and number of fruits umbellet^{-1} (-0.144).

A strong positive correlation of umbels plant^{-1} was seen with seed yield plant^{-1} (0.830), number of umbellets umbel^{-1} (0.608), number of fruits umbel^{-1}(0.422) and number of fruits umbellet^{-1}(0.306). The number of umbellets umbel^{-1} recorded a significant positive association with seed yield plant^{-1} (0.564), number of fruits umbel^{-1} (0.404) and number of fruits umbellet^{-1} (0.352). The number of fruits umbellet^{-1} had a positive and significant correlation with seed yield plant^{-1} (0.291), number of fruits umbel^{-1} (0.291) and 1000-seed

Table 2. Estimates of genotypic and phenotypic correlation coefficients for yield and its contributing characters in coriander (2nd year).

Character		Chlorophyll content 60 DAS	No. of primary branches	No. of fruiting nodes	Days to 50% flowering	Days to 80% maturity	No. of umbels plant^{-1}	No. of umbellets umbel^{-1}	No. of fruits umbellet^{-1}	No. of fruits umbel^{-1}	Diameter of fruit (mm)	Vegetative yield (kg)	1000-seed weight (g)	Seed yield plant^{-1} (g)
Plant height at maturity	G	0.415	0.093	0.039	0.165	0.158	0.071	0.149	0.042	-0.008	-0.029	0.128	0.001	0.245
	P	0.414**	0.087	0.039	0.165*	0.158*	0.071	0.096	0.033	-0.005	-0.015	0.121	0.001	0.238**
Chlorophyll content 60 DAS	G		0.363	0.115	0.289	0.254	0.186	0.184	0.184	0.166	-0.006	0.248	0.010	0.359
	P		0.333 **	0.116	0.287**	0.252**	0.182**	0.121	0.149*	0.092	-0.002	0.230**	0.010	0.344**
No. of primary branches	G			0.519	0.016	-0.025	0.567	0.649	0.556	0.550	0.501	-0.136	-0.124	0.634
	P			0.468**	0.015	-0.021	0.517**	0.403**	0.395**	0.264**	0.287**	-0.117	-0.097	0.573**
No. of fruiting nodes	G				0.109	0.105	0.972	0.950	0.417	0.732	0.012	-0.037	-0.067	0.843
	P				0.107	0.103	0.951**	0.609**	0.316**	0.409**	0.019	-0.039	-0.076	0.805**
Days to 50% flowering	G					0.991	0.084	0.187	-0.165	-0.110	-0.066	0.324	-0.281	0.295
	P					0.988**	0.082	0.118	-0.129	-0.063	-0.043	0.306**	-0.272**	0.285**
Days to 80% maturity	G						0.075	0.183	-0.181	-0.110	-0.093	0.323	-0.277	0.293
	P						0.074	0.122	-0.144*	-0.056	-0.055	0.306**	-0.268**	0.283**
No. of umbels plant^{-1}	G							0.931	0.398	0.750	0.082	-0.020	-0.095	0.868
	P							0.608**	0.306**	0.422**	0.043	-0.019	-0.093	0.830**
No. of umbellets umbel^{-1}	G								0.588	0.901	0.122	0.004	0.047	0.897
	P								0.352**	0.404**	0.029	0.009	0.011	0.564**
No. of fruits umbellet^{-1}	G									0.818	0.015	-0.177	0.263	0.334
	P									0.291**	-0.042	-0.103	0.190**	0.291**
No. of fruits umbel^{-1}	G										-0.162	0.047	0.027	0.669
	P										-0.011	0.008	0.032	0.320**
Diameter of fruit (mm)	G											-0.319	-0.151	0.113
	P											-0.199**	-0.096	0.090
Vegetative yield (kg)	G												-0.167	0.157
	P												-0.137*	0.142*
1000-seed weight (g)	G													-0.153
	P													-0.140*

*,**Significant at 5 and 1% level, respectively.

weight (0.190). The number of fruits umbel^{-1} exhibited a significant positive association with seed yield plant^{-1} (0.320) while it was negatively associated with vegetative yield plot^{-1} (-0.199). Vegetative yield plot^{-1} had a significant positive association with seed yield plant^{-1} (0.142) but a negative correlation with 1000-seed weight (-0.137). Thousand-seed weight was also negatively correlated with seed yield plant^{-1} (0.140).

On the basis of pooled data, coefficients of correlation of yield and its component traits has been depicted in Table 3. Seed yield plant^{-1} exhibited a positive and significant correlation with number of fruits umbellet^{-1} (0.233) but it was negatively correlated with days to 50% flowering (-0.286), days to 80% maturity (-0.280) and vegetative yield plot^{-1} (-0.141). Plant height at maturity had a significant positive association with chlorophyll content at 60 DAS (0.409), 1000-seed weight (0.252), days to 50% flowering (0.157) and days to 80% maturity (0.155). Chlorophyll content at 60 DAS showed positive and significant association with number of primary branches plant^{-1} (0.355), 1000-seed weight (0.349), days to 50% flowering (0.279) and 80% maturity (0.249), vegetative yield plot^{-1} (0.232), number of umbels plant^{-1} (0.184) and number of fruits umbellet^{-1} (0.173). Number of primary branches plant^{-1} exhibited a positive significant correlation with 1000-seed weight (0.565), number of umbels plant^{-1} (0.490), number of fruiting nodes plant^{-1} (0.475), number of fruits umbellet^{-1} (0.411), number of umbellets umbel^{-1} (0.401), number of fruits umbel^{-1} (0.327) and diameter of the fruit (0.310).

Strong positive and significant association of number of fruiting nodes plant^{-1} was observed with number of umbels plant^{-1} (0.944), 1000-seed weight (0.822), number of umbellets umbel^{-1} (0.572), number of fruits umbel^{-1} (0.454) and number of fruits umbellet^{-1} (0.279). Highly significant and positive correlation of days to 50% flowering with days to 80% maturity (0.986), vegetative yield plot^{-1}(0.309), and 1000-seed weight (0.297) was noted while a negative correlation with number of fruits umbellet^{-1} (-0.146) was observed. Days to 80% maturity had a positive and significant correlation with vegetative yield plot^{-1} (0.298), 1000-seed weight (0.298) and number of umbellets umbel^{-1} (0.138); it was negative with number of fruits umbellet^{-1} (-0.156).

A highly positive association of number of umbels plant^{-1} was recorded with 1000-seed weight (0.836), number of umbellets umbel^{-1} (0.552), number of fruits umbel^{-1} (0.451) and number of fruits umbellet^{-1} (0.237). Number of umbellets umbel^{-1} showed a positive and significant association with 1000-seed weight (0.523), number of fruits umbel^{-1} (0.482) and number of fruits umbellet^{-1} (0.320). Number of fruits umbellet^{-1} expressed a positive significant correlation with number of fruits umbel^{-1} (0.401) and 1000-seed weight (0.232). Thousand seed weight was also positively correlated with number of fruits umbel^{-1} (0.372) while diameter of the fruit was negatively correlated with vegetative yield plot^{-1} (-0.205).

DISCUSSION

The correlation coefficient is a statistical tool that is used to find out the degree (strength) and direction of relationship between 2 or more variables. A positive value shows that changes in 2 variables are in the same direction i.e., the value of 1 variable is associated with the other variable, whereas a negative value shows that the movements of the variables are in the opposite direction i.e., the high value of one variable is associated with the low value of the other.

The association between 2 variables that can be directly observed is called phenotypic correlation. It includes both genotypic and environmental effects and therefore it differs under different environmental conditions. Genotypic correlation is the inherent association between two variables. This type of correlation is more stable and is of paramount importance for a plant breeder to bring about genetic improvement in one character by selecting the other character of a pair that is genetically correlated. Environmental correlation is entirely due to environmental effects and error variance. This is not of much importance as it is not heritable and stable. In the present investigation, correlation coefficients were estimated between yield and its components at

Table 3. Estimates of genotypic and phenotypic correlation coefficients for yield and its contributing characters in coriander (pooled data).

Character		Chlorophyll content 60 DAS	No. of primary branches	No. of fruiting nodes	Days to 50% flowering	Days to 80% maturity	No. of umbels plant^{-1}	No. of umbellets umbel^{-1}	No. of fruits umbellet^{-1}	No. of fruits umbel^{-1}	Diameter of fruit (mm)	Vegetative yield (kg)	1000-seed wt. (g)	Seed yield plant^{-1} (g)
Plant height at maturity	G	0.413	0.133	0.050	0.158	0.154	0.083	0.172	0.053	0.079	-0.045	0.119	0.255	0.018
	P	0.409**	0.125	0.050	0.157*	0.155*	0.079	0.108	0.045	0.039	-0.030	0.114	0.252**	0.017
Chlorophyll content 60 DAS	G		0.387	0.121	0.282	0.253	0.190	0.218	0.201	0.209	-0.013	0.240	0.362	0.038
	P		0.355**	0.121	0.279**	0.249**	0.184**	0.135	0.173*	0.108	0.001	0.232**	0.349**	0.037
No. of primary branches	G			0.511	0.011	-0.019	0.540	0.661	0.532	0.650	0.464	-0.147	0.615	-0.075
	P			0.475 **	0.014	-0.013	0.490**	0.401**	0.411**	0.327**	0.310**	-0.126	0.565**	-0.066
No. of fruiting nodes	G				0.115	0.113	0.968	0.910	0.334	0.799	0.008	-0.042	0.847	-0.043
	P				0.115	0.114	0.944**	0.572**	0.279**	0.454**	0.016	-0.042	0.822**	-0.047
Days to 50% flowering	G					0.991	0.105	0.181	-0.164	-0.087	-0.067	0.318	0.305	-0.288
	P					0.986**	0.103	0.122	-0.146*	-0.054	-0.047	0.309**	0.297**	-0.286**
Days to 80% maturity	G						0.100	0.192	-0.178	-0.082	-0.093	0.308	0.309	-0.280
	P						0.096	0.138*	-0.156*	-0.040	-0.065	0.298**	0.298**	-0.280**
No. of umbels plant^{-1}	G							0.884	0.288	0.754	0.061	-0.028	0.870	-0.088
	P							0.552**	0.237**	0.451**	0.035	-0.025	0.836**	-0.082
No. of umbellets umbel^{-1}	G								0.566	0.983	0.125	0.036	0.880	0.083
	P								0.320**	0.482**	0.028	0.021	0.523**	0.047
No. of fruits umbellet^{-1}	G									0.861	-0.002	-0.143	0.256	0.283
	P									0.401**	-0.015	-0.112	0.232**	0.233**
No. of fruits umbel^{-1}	G										-0.158	0.013	0.751	0.128
	P										-0.023	-0.007	0.372**	0.072
Diameter of fruit (mm)	G											-0.27	0.111	-0.129
	P											-0.205**	0.084	-0.095
Vegetative yield (kg)	G												0.140	-0.149
	P												0.132	-0.141*
1000-seed weight (g)	G													-0.130
	P													-0.125

*, **Significant at 5 and 1% level, respectively.

genotypic, phenotypic and environmental levels to identify the interrelationship among different traits. It was found that genotypic correlation coefficients were higher than phenotypic ones because of the masking effect of genotypes for the expression of characters.

Seed yield plant^{-1} exhibited a positive and significant correlation with number of fruits umbel^{-1} but showed negative correlation with days to 50% flowering and 80% maturity. The results confirm the findings of Ali *et al.* (2004), Prabhu and Balakrishnamoorthy (2005), Singh *et al.* (2006), and Dalkani *et al.* (2011). In contrast, Selvarajan *et al.* (2002) observed a positive association of seed yield with days to 50% flowering.

Plant height at maturity had a significant positive association with chlorophyll content at 60 DAS, 1000-seed weight, days to 50% flowering and 80% maturity. The results are in close proximity with those of Tripathi *et al.* (2000), Selvarajan *et al.* (2002), Prabhu and Balakrishnamoorthy (2005) and Dalkani *et al.* (2011). Chlorophyll content at 60 DAS showed positive and significant association with number of primary branches plant^{-1}, 1000-seed weight, days to 50% flowering and 80% maturity, vegetative yield plot^{-1}, number of umbels plant^{-1} and number of fruits umbellet^{-1}.

The number of primary branches plant^{-1} exhibited a positive significant correlation with 1000-seed weight, number of umbels plant^{-1}, number of fruiting nodes plant^{-1}, number of fruits umbellet^{-1}, number of umbellets umbel^{-1}, number of fruits umbel^{-1} and diameter of the fruit. The present findings agree with earlier results of Rajput *et al.* (2004), Singh *et al.* (2007), Singh and Prasad (2006) and Singh *et al.* (2007). Strong positive and significant association of number of fruiting nodes plant^{-1} was observed with number of umbels plant^{-1}, 1000-seed weight, number of umbellets umbel^{-1}, number of fruits umbel^{-1} and number of fruits umbellet^{-1}. Singh *et al.* (2007) observed similar results.

Highly significant and positive correlation of days to 50% flowering with days to 80% maturity, vegetative yield plot^{-1}, and 1000-seed weight was noted and a negative correlation with number of fruits umbellet^{-1} was observed. These results support the findings of Ali *et al.* (2004), Rajput *et al.* (2004), and Prabhu and Balakrishnamoorthy (2005). However, Singh *et al.* (2007) observed a positive association of days to 50% flowering with number of fruits umbel^{-1} and umbellet^{-1}.

Days to 80% maturity had a positive and significant correlation with vegetative yield plot^{-1}, 1000-seed weight, and number of umbellets umbel^{-1}; it had negative correlation with number of fruits umbellet^{-1}. The same results were reported by Rajput *et al.* (2004) and Prabhu and Balakrishnamoorthy (2005). Highly positive association of number of umbels plant^{-1} was recorded with 1000-seed weight, number of umbellets umbel^{-1}, number of fruits umbel^{-1}, and number of fruits umbellet^{-1}. Singh *et al.* (2004, 2007) found quite similar results.

Number of umbellets umbel^{-1} showed a positive and significant association with 1000-seed weight, number of fruits umbel^{-1}, and number of fruits umbellet^{-1}. The present findings confirmation the observations of Prabhu and Balakrishnamoorthy (2005); Singh and Prasad (2006) and Dalkani *et al.* (2011). Number of fruits umbellet^{-1} expressed a positive significant correlation with number of fruits umbel^{-1} and 1000-seed weight. Thousand-seed weight was also positively correlated with number of fruits umbel^{-1}, whereas fruit diameter was negatively correlated with vegetative yield plot^{-1}, results in close proximity to the findings of Singh *et al.* (2007).

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