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BREEDING AND BIOTECHNOLOGICAL APPROACHES FOR IMPROVING THE PRODUCTIVITY OF SUNFLOWER HYBRIDS

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SUMMARY

Sunflower has become a major edible oil seed crop of India in less than 30 years of its introduction. This crop is ideally adapted to both irrigated and rainfed farming. Recently, considerable progress has been made in the development of commercial hybrids in India. In our sunflower improvement program, both conventional breeding and plant tissue culture techniques were followed to develop hybrids with high seed yield and oil content. Broad based B-gene and R-gene pool populations were developed to derive potential inbred lines. Numerous traits, plant vigor, self-fertility, early maturity, disease resistance, oil content and combining ability were considered for selection during inbred development. B- and R-gene pools were used as effective testers in the top crosses for screening a large number of inbred lines. The combining ability of out advanced B-gene and R-gene pool- derived inbreds was predicted by crossing them with selected restorer and cytoplasmic male sterile (CMS) lines, respectively. The potential B-gene pool-derived inbreds were converted into CMS lines in one and half to two years time by conventional backrossing and supplemented with embryo culture technique. Further, in immature embryo culture the F_1 hybrids were induced to flower in the test tubes and produced embryo like structures in pollen sacs of *in vitro* flowers.

INTRODUCTION

Sunflower was introduced in India in 1968. It developed into one of the major oil seed crops of the country in less than 30 years. Currently, sunflower is being cultivated in over 1.5 m hactares. It is adapted to different regions, seasons and cropping systems of India. Sunflower oil in the country commands higher price than the traditional peanut and rape seed oils.

In early years of introduction, the sunflower cultivation suffered setback due to poor seed setting, low yield and oil content and disease susceptibility of open-pollinated cultivars introduced from Russia and Canada. These problems were soon overcome by developing early maturing, higher yielding and disease resistant hybrids. The hybrids also reduced the problem of dependence on insect pollination because of their inherent high self-fertility. In the last few years, there has been wide spread cultivation of sunflower hybrids throughout the country. However, the productivity of these hybrids is lower than compared to hybrids recently introduced from USA, Canada and Australia. In our sunflower research program, conventional breeding and biotechnological techniques are being followed to develop potential inbreds relatively at shorter time for hybrid breeding and finally improve the productivity of hybrids.

MATERIALS AND METHODS

A composite was developed from twelve populations, Morden, CO-1, CO-2, SUF-6, SUF-7, GP-2, GP-26, GP-232, GP-237, GP-435, EC-19087 and EC-250285. The first three populations were commercial cultivars. SUF-6 and SUF-7 were the self-fertile populations. Equal quantities of seed of these populations were bulked and grown in isolation. The undesirable plants were removed before flowering. Intercrossing was done by hand pollination. All the plants were harvested and seed was bulked to form a composite population. From the composite population, separate maintainer (B) and restorer (R) gene pools were synthesized and used to develop inbred lines. The potential inbred lines were converted into cytoplasmic male sterile (CMS) and restorer lines following conventional breeding and biotechnological procedures.

RESULTS AND DISCUSSION

BREEDING APPROACHES:

Synthesis of maintainer (B) and restorer (R) gene pools

In India, most hybrids were developed from limited CMS and restorer lines introduced from the U.S.A. and Canada. Several Indian sunflower hybrid research programs are still lacking the major breeding emphasis on developing inbred lines and converting them into CMS and restorer lines. Hybrids produced from such lines will have better adaptability to varied agro-climatic conditions of India., Open-pollinated cultivars or composites of planned crosses are commonly utilized as source populations to derive inbred lines. Such populations possess self-incompatiable alleles for ensuring cross pollination. But the sunflower breeders emphasize the selection for self-fertility especially when inbred lines are used in the production of hybrids (Fick 1978). The inbreds that produce large quantities of seed upon self-pollination are not only easy to maintain but also produce self-fertile hybrids. The built-in self-fertility of hybrids may result in high seed yield when insect pollination is less than minimum. It would be easy to develop self-fertile inbreds when there is high frequency of self-fertile alleles in a source population. Separate maintainer (B) and restorer (R) gene pools were synthesized in our breeding program from selected high self-fertile lines. A composite population was first developed from two self-fertile and ten diverse populations. Phenotypically vigorous plants in the composite population were selfed and selected for self-fertility and oil content. Half the seeds of selected plants was used to Braise S_1 progenies. The S_1 progenies were evaluated for self-fertility, oil content and disease resistance. Simultaneously, the lines were crossed with male sterile stock for the presence of maintainer or restorer genes. The reserve seed of selected plants, which possess maintainer genes and single heads, was composited and intercrossed in isolation to constitute B-gene pool. Similarly R-gene pool was constituted from selected plants possessing restorer genes. Further genetic upgradation of these gene pools could be done following intra- and inter-population recurrent selection program (Virupakshappa, 1987).

The data in Table 1 illustrates that B- and R-gene pools had higher autogamy percentage and seeds per plant than the composite population. Evidently, the frequency of self-fertile alleles was increased in B- and R-gene pools. The inbred lines derived from these gene pools also showed high self-fertility and number of seeds per plant.

Development of inbred lines

The B- and R-gene pools, were used as base populations for developing inbred lines. In each season, 2000 - 5000 phenotypically vigorous plants were selfed at the time of flowering using muslin cloth bags. Further, the selection was made at harvest time for self-fertility, low hull content, early maturity and disease resistance. Usually 15-20% plants were retained to raise plant-to-row progenies in the following season. The inbreeding was continued with selection among and with progeny rows. In sunflower, several progenies show uniformity in S_3 generation itself. The combining ability tests were performed in S_3 for rejecting a large number of lines. Over 250 inbred lines have been developed in less than three years utilizing two to three crop seasons in an year. Substantial variability has been observed among inbreds for maturity, plant height, number of seeds per plant, oil content and self fertility. The autogamy ranged from 42 to 94%. Since most inbred lines produced more than 180 seeds per plant, ther was no problem of maintaining them. The oil content among inbreds varied from 34 to 48%.

Evaluation of inbreds for combining ability

Early generation selection for combining ability was done in S_3 generation using inbred x population tester crosses. The R-gene pool was used as tester for evaluating inbred lines derived from B-gene pool. Similarly, B-gene pool was the tester for R-gene pool derived inbred lines. The inbreeding was continued in *S_3 progenies showing high combining ability. In advanced generation of inbreeding, specific tests such as inbred x inbred crosses were performed for predicting the general and specific combining abilities of inbreds. Widely adaptable CMS lines already available in our program were used as testers for evaluating the productivity of lines derived from R-gene pool, while the restorer lines were used as testers for determining the productivity of B-gene pool derived lines. In sunflower, the genic male sterile plants frequently appear in restorer lines. The application of gibberllic acid (GA) spray can also induce sterility. Therfore, the problem of hand emasculation in restorer x inbred line test crosses has been overcome with the availability of genic male sterility or induced male sterility by GA.

Converstion of inbred lines into CMS and restorer lines

The R-gene pool derived inbred lines with high combining ability was readily used in the synthesis of hybrids. However, the productive inbred lines deveoped from B-gene pool were converted into CMS lines by back cross method. Embryo culture technique supplemented to back crossing to hasten the conversion program.

BIOTECHNOLOGICAL PROCEDURES

Embryo culture

Conventional backcrossing is simple and efficient procedure to convert the given line into CMS line but time consuming. Supplementation of embryo culture with conventional backcrossing can hasten the conversion process. In sunflower, only two to three generations per year are possible due to 40-45 days of seed dormancy. Using embryo culture 4-5 generations can be raised in a year.

Seeds were collected from twenty specific crosses involving CMS stock and maintainer lines after ten days of pollination. The maintainer lines were selfed and the seeds were collected after ten days of anthesis. After surface sterilization, the embryos were aseptically disected out and cultured on Murashige and Skoog's (1962) medium containing 3% sucrose with or without growth regulators. The cultures were maintained at $25 \pm 2^{\circ}$ C under 16-8 hour light dark regime. Cotyledons turned green and roots elongated in about two days. Healthy 3-4 cm long plantlets were formed in about a week. Frequency of plantlet formation ranged between 75-100% and survival on transfer was 65-80%. The plantlets reached flowering stage in about 35-40 days bearing smaller flowers. Howevewr, the seeds derived were viable enabling us to proceed for next generation. Using the above procedure, we could cut short the time required to raise F_1 lines by about 50 days in between generations demonstrating the effective use of embryo culture for CMS conversion program.

Imature embryo culture

Another aspect we studied was to assess the morphogenetic potential of immature hybrid embryos for their possible practical application in the breeding programme. Immature hybrid embryos were cultured in various media excising them after 3,5,7,9 and 11 days of pollination. In auxin containing media, hybrid embryos of all stages have shown callusing. In rest of the media, multiple adventitious bud shoot induction was observed. While five day old embryos had shown 56% response with 1.36 shoot buds/embryo whereas in 9 day old ones 2.36 buds were produced at 84% frequency. In order to allow the multiple shoots to grow in the absence of apical dominance, the terminal buds of the germinated and growing embryos were decapitated and transferred to media with 2.5 mg/1 BAP. A proportion of adventitious bud meristems showed floral mode of development. Very clear formation of florets was visible though some of the buds remained either vegetative or intermediate. Five day old embryos showed higher frequency of flower formation ranging from 28-36 % whereas those from 9 day old and others exhibited 18% or lower fequency. Embryo like bodies upto 24 celled stage were observed along with pollen in the pollen sacs structures of which tallied with description of *in vitro* embryos (Newcomb, 1972).

PRODUCTIVITY OF NEW HYBRIDS

Twenty CMS lines and 60 restorer lines of early to medium maturity were developed at our centre. It is posssible for us to synthesize 200-250 new hybrids from these lines in every season. The hybrids can be tested in two seasons, monsoon season (June-September) and summer season (January-April) every year. In 1991-92, 240 hybrids were evaluated in two-row, 4 m length, replicated plots with two repeated checks for two seasons. The popular hybrids, MSFH-8 and MSFH-17, developed by Maharashtra Hybrid Seed Co. Ltd were used as checks. In the monsoon season, 38 hybrids were superior to checks for seed yield. Fifty two hybrids, however, showed higher seed yield when compared to checks in summer season. Only eleven hybrids outyielded checks in both seasons (Table 1). The data clearly indicated seed yeild improvement of hybrids. This could be attributed to the potential of inbred lines used for hybrid development. Besides the seed yield, improvement was made with hybrids for oil content, earliness and self-fertility.

CONCLUSIONS

Separate B- and R-gene pools with high frequency of self-fetility alleles were synthesized from diverse populations and used to develop CMS and restorer lines respectively. These gene pools yielded highly potential inbred lines for producing hybrids for high seed yield and oil content. Further, it was possible to convert inbred lines into CMS lines in one and half to two years time by conventional back cross method supplemented with embryo culture.

Table 1 : Autogamy (%) and seeds per plant in composite, B - and R- gene populations

Population	Autogamy (%)		Seeds per plant	
	Mean ± SE	Range	Mean ± SE	Range
Composite	31.7 ± 4.0	0-89	161.5 ± 28.7	0 - 397
B - gene pool	46.6 ± 4.3	16-92	224.9 ± 19.8	43 - 506
R - gene pool	49.4 ± 3.7	29-97	265.3 ± 16.2	67 - 459
Inbreds	63.9 ± 2.9	42-94	293.3 ± 21.5	189 - 634

Table 2 : Performance of SPIC hybrids outyielding checks in two seasons, 1991-92.

	Pre-rainy		Post rainy	
Hybrids	Seed yield (q/h)	Oil (%)	Seed yield (q/h)	Oil (%)
SPIC hybrids				
91-20	28.2	40.4	29.7	39.8
91-57	37.6	39.6	38.0	41.0
91-68	34.3	42.6	31.1	44.2
91-77	29.0	35.8	30.2	38.5
91-88	27.5	36.2	22.3	38.2
91-89	27.6	34.9	25.6	33.7
91-99	29.8	43.1	26.0	41.8
91-116	31.8	37.9	27.2	39.0
91-125	30.9	37.3	21.0	35.4
91-215	29.7	39.4	31.0	38.8
91-226	30.7	41.6	26.0	39.4
Checks				
MSFH-8	23.7	36.3	20.1	38.3
MSFH-17	25.1	32.9	19.2	35.1

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