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Somaclonal Variation in Microsperma Lentil (*Lens culinaris* Medik)

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Abstract

Shoot apices of Masoor-85 seedlings (3-4 days old) induced proliferated callus masses under dark conditions, were induced to proliferate callus masses. The callus was induced under dark conditions in MS medium containing K (10 mg/l), GA (1 mg/l) with 5-10 percent lentil seed extract. The shoots were regenerated by transferring the callus culture in light. The shoots were cut and rooted to have plantlets that were finally grown in the field in winter season. These plants were Ro generation. The first selections were made from Ro and seeds were collected. These seeds were grown for R1 generation, whose seeds were selected for R2 generation and so on. Some selected variants were stable till AS generation and were called somaclonal. Variants for more pods and large seed were selected.

Introduction

Lentil, is one of the oldest food crops which originated in the fertile crescent of the near East. It dates back to the beginnings of agriculture itself. It is grown on marginal and unirrigated land in rabi (winter) season. It is self pollinated grain legume that belongs to Fabaceae. The primary product of lentil crop is the mature seed. It is nutritionally very rich (Anigbogu, 1997) and an important caloric and protein rich source (Singh and Singh, 1994). Abu-Shakra and Tannous (1981) stated that the lentil protein content was comparable to that of faba bean and it was higher than that of chickpea and more than double that of wheat.

More yield is a commercially much desired character. Yield per plant is governed by number of pods per plant and seed size (Ali *et al*., 1997). The number of pods per plant is strongly regulated by genotypic characters (Rahman and Sarker, 1997).

Somaclonal variation was defined by Larkin and Scowcroft (1981) as the genetic variation displayed in tissue culture regenerated plants and their progeny. Differentiated plant tissues are the source of callus *in vitro*. Gross genomic changes can occur during differentiation *in vitro* (D'Amato and Bayliss, 1985). If such cells are cultured *in vitro* these changes will be apparent in the regenerated plants. Somaclonal variation is a kind of induced diversity in a given crop. Like plant breeding which primarily involves creation of variability and evaluation and selection of variants, somaclonal variation is also important factor in crop improvement (Zuk, 1997).

The objective of this study was to compare the plant characteristics of lentil cultivar Masoor-85 with those plants regenerated from its callus culture and to select some useful somaclonal variants for seed size and for more pods, if any and also compare with subsequently seed generated progeny populations of R1, R2, R3, R4 and R5.

Materials and Methods

Apical meristem explants from three to four days seedlings of lentil cultivar Masoor-85 were grown in MS medium (Murashige and Skoog, 1962) containing K (10 mg/l), GA (1 mg) with ±5-10 percent lentil seed extract; in dark at 21 1°C. The callus responsive explants were subcultured twice in the same medium for increasing the callus masses. Each subculture interval was 4 weeks. When these calli were placed in the same medium in light, the regeneration started as green dots and ultimately shoots formed on the surface of the calli. The shoots were cut from bases that were dusted with rooting powder (IBA 0.008% + NAA 0.002% in simple talc) placed on the aluminum foil and then transferred to soil in pots. The pots were covered with polythene for at least 15 days and shoots were sprayed with water to retain humidity until new leaves appeared. The rooted shoots grew well; after flowering and fruiting, the pods were collected and seeds were obtained. The seeds were germinated and studied for their progenies. The progenies under field test were *in vitro* regenerated plants (Ro) and the first (R1), the second (R2), the third (R3), the fourth (R4) and the fifth (R5) were plants from seeds harvested from successive generations. All progenies including population of Masoor-85 (control) were grown during the rabi (winter) seasons in fields at NIAB, Faisalabad. The populations were sown in the first week of November each year, from 1991 to 1996 and harvested in the last week of April, from 1992 to 1997. The plants were initially selected from Ro. Every year sixty seeds, of the selected plant from various progenies, were sown in rows, with row to row distance of one foot. For 10 rows of the selected plants, there were two rows of the control (Masoor-85) plants.

$$\text{H.I.} = \frac{\text{Grain yield of plant}}{\text{Total weight of plant}} \times 100$$

The plants that had different characters from the control plant and had different overall look, were selected among various populations for further progeny study. The characters like, plant type, plant height, nodes on main axis, branches per plant, pods per plant, grain yield (gms), H.I. (percent), 100 seed weight (gms), maturity were noted.

Plants of R0 to R5 populations showed much variations within the populations. These variations may have come through callus formation from which the plants were originally derived and through shoot organogenesis. The selections were made for large seed size, more podded and

small seed size variants, particularly. For yield assessment of the selected lines, three sets of small plots (50 plants/plot, plant to plant distance 6 inches) were cultivated, each for large size seed variant, more podded variant, small seed size variant and control (Masoor-85). Finally, the yield assessment was made.

In the other experiment, three plots (9 × 25 ft.) were sown, for the selected variants; large seeded variant, more podded variant and parent Masoor-85. The pooled yield of each variants was compared.

Results

There were much variations in plant shape and morphological structure in populations of Ro-R5 progenies. (data not provided). The number of pods per plant, 100-seed weight, percent H.I. are important yield contributing characters (Table 1). The maximum number of pods in one of the plant was 2630 in progeny (R4) and another plant with the lowest number of pods in the same progeny had 138 pods. The maximum number of pods in one of the plant was 480 in progeny (Ro) and in the same progeny had the lowest number of pods as 40. The highest 100 seed weight for one the plant was 3.8 in progeny (R5) and in the same progeny another plant showed 100 seed weight as 0.73. The highest harvest index was for a plant in progeny (R5), 116.78 and in the same progeny the lowest harvest index for another plant was 10.71.

There was continuous selection of plants within the same selected plant progenies. The maximum and minimum numbers are given in the table only, to give an idea about variation induced. A more podded line as compared to the control (Masoor-85) was selected from a plant that originally produced 2,630 pods in R4. But the somaclone line with more number of pods per plant had pod shedding problem at maturity time. Why it did not retain all pods till full physiological maturity of plants, needs further testing. There were plants which were having 100-seed weight more than 2.11 gm (more than that of control 1.54 gm.). From the large seeded plant, a somaclone line was derived whose 100 seed weight was above 3 gms. There was also a small seeded line which continuously produced small seeds in some plants of subsequent progenies. However small seed size trait was not stable. Only one plant out of thirty in R4 gave maternal seed size. Small seeds were of dark colour as compared to Masoor-85. One plant that produced small seeds, its progeny produced 50 per cent small seed size plants in R5. The average 100-seed weight of small sized seeds variant was 1.0 g, only (data not given).

somaclone variants with large seed size and those with more pods were important products of this study. Their characters remained stable in progenies studied. However the small size seed variant was unstable even upto R5. There were always plants in the small size seed somaclone progeny which produced seeds that varied in sit considerably. Promising characters of some somaclon variants are given in Table 2. Height was maximum (40 cms) in case of more podded variant but the number of nodes on main axis was maximum (19) in case of large seeded variant while nodes were minimum (13) in small seeded variant. Primary and secondary branches were maximum (15) in large seeded followed by more podde' variant (13). The small seeded variant had more or less similar number of primary and secondary branches (7) as in control (8). The biological yield (61 gms), the grain yield (30.62 gms) per plant and the 100-seed weight (3.30 gms) was maximum in large seeded variant which matured by the end of April when the temperatures were high. While the other two variants and the control matured by the mid-April. The more podded variant bore 813 pods and also had fairly large size seeds (100 seed weight = 3.2 gms). Three sets of 50 plants each belonging to larg seeded, more podded variant, small seed line, final selection from R4 progeny and control (Masoor-85) gave average yield of 457 gm, 365 gm, 68 gm and 234 gm per 50 plant respectively. The large seed line increased the yield by 85 percent, more podded variant increased the yield by 55 percent and small seeded lines decreased the yield by 71 percent as compared to control (Masoor-85).

Table 1: Variation in yield characters of Callus regenerated progenies of Masoor-85 (c)

		Pods/Plant (No.)	100 Seed weight (g)	Grain yield/ plant (g)	Biol. Yield/ plant (g)	%H.I.
RO	Maximum	480.00	3.00	23.27	41.00	48.12
	Minimum	40.00	1.20	4.13	11.21	13.45
R1	Maximum	560.00	2.32	24.31	48.00	54.41
	Minimum	103.00	0.86	8.12	16.10	23.28
R2	Maximum	1350.00	3.16	40.26	105.10	85.63
	Minimum	68.00	1.82	4.32	19.00	20.16
R3	Maximum	1237.00	2.78	27.70	71.00	77.00
	Minimum	107.00	1.94	14.21	42.00	10.82
R4	Maximum	2630.00	2.47	21.47	52.00	70.44
	Minimum	138.00	1.20	5.20	19.50	12.61
R5	Maximum	765.00	3.80	19.90	80.70	116.78
	Minimum	175.00	0.73	3.53	10.00	10.71
Control	Maximum	697.00	2.11	37.40	76.00	55.75
	Minimum	67.00	0.62	2.19	19.50	10.07

A plant with maximum and an other with minimum number of pods within progeny is described and no. is for of characters

Table 2: Agronomic characters of selected Somaclone variants

Variant character	M-85 (check)	Small seeded	Large seeded	More podded
Plant type	Spreading bushy	Erect	Erect	Bushy erect
Plant Height (cms)	25.00	27.00	36.00	40.00
Nodes on main axis	17.00	13.00	19.00	18.00
Branches per plant	8.00	7.00	15.00	13.00
Pods per plant	213.00	208.00	512.00	813.00
100 seed weight (gms)	1.54	1.20	3.30	3.24
Grain yield (gms)	14.50	11.05	30.62	28.84
Biological yield (gms)	46.00	30.00	61.00	55.00
H.I. (percent)	31.52	36.83	50.19	52.43
Maturity	Mid-April	Mid-April	End of April	Mid-April

The other experiment was with R5. Three plots 19x25 ft.) were sown, one each for large seed variant, more pod variant and Masoor-85 (control). There were ten rows in each plot and there were 51 plants per row. The pooled yield was 4.6 kg for large seed variant, 5.2 kg for more pod variant and 1.9 kg for Masoor-85. Although there were losses during manual harvesting but the ground fallen seeds were not collected and considered.

The large seed size variant whose seed colour is lighter as compared to Masoor-85 had mostly good quality plant type except at maturity times, some of the pods shattered. The pod shattering was at basal portions of plant. More podded variant had most of the desirable characteristics but its lower mature pods dropped down before harvesting. It had pod shedding problem perhaps because of more number of pods it bore.

Discussion

The objective of the present study was to compare the characters of the *in vitro* regenerated plants and their progenies with those of Masoor-85 populations and make selections for somaclonal variants. According to Larkin and Scowcroft (1981), a somaclonal variant is obtained when an explant is subjected to a tissue culture cycle. One of the potential benefits of somaclonal variation in plant genetic manipulations was seen to be the opportunity to create additional genetic variability in co-adapted, agronomically useful cultures without the need to resort to hybridization or the production of transgenic plants. Tissue culture method is clearly a mutagenic procedure (Phillips *et al*., 1990) and the main source of genetic variability were gene mutations and recombinations. Changes detected in the sexual progeny of regenerated plants are often considered to be stable and contrasted with the epigenetic traits sometimes detected in tissue culture or primary regenerations. In this study, seed grown population of Masoor-85 and regenerated populations were compared for vegetative and yield characters and selections were made for vegetative and yield characters, uniform physiological maturity, erect plant type more number of pods, larger seed size and lighter seed colour, small seed size etc.

Somaclones comprises three classes of variation (1) Heritable, Stable (Non-reversible) changes (2) Heritable unstable (reversible) changes and (3) Non-heritable, transient (epigenetic) changes. In this work of microsperma lentil more podded variant and large seed size was heritable character. However, small seed size was unstable in progeny. The small seed size was obtained in plants till R5 generation. However large seed size and lighter seed colour was most stable character in the progenies of the selected plants and was the most important somaclonal variant of this study. Yield per plant is governed by number of pods per plant and seed size (Rahman and Sarker, 1997).

Since little work is available on lentil cell and tissue culture, there is no such report as somaclonal variation in lentil. The improvement of yield by large seed size and more podded variants in this study is an important contribution of lentil tissue culture.

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