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MORPHOLOGICAL AND CYTOLOGICAL VARIATION IN SECOND SOMACLONAL GENERATION OF BARLEY

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ABSTRACT

Somaclonal variation was studied in SC_2 generation of in vitro regenerated resistant plants of barley cv. Dissa. Two seedling mutants, internodeless and freak narrow leaf were obtained. Nucleus was multilobed in most of the cells in the somaclonal line which showed internodeless mutant. 50% of in vitro selected somaclonal lines showed stable transmission of resistance to *Helminthosporium sativum*.

Key words: Somaclonal variation, *Helminthosporium sativum*, *Hordeum vulgare*, nuclear abnormalities.

Extensive research work has proved that somaclonal variation is much higher than that generated by spontaneous mutation. Early maturity, higher yield, improved nutritional quality, disease resistance, etc., have been reported in somaclonal generations of different crops. Barley plants resistant to *Helminthosporium sativum* obtained through in vitro selection of callus have been reported by Chawla and Wenzel [1]. The progenies of these in vitro regenerated plants (SC_2 generation) were tested for stable transmission of resistance to *H. sativum*, variation for morphological characters and cytological abnormalities in somaclonal lines.

MATERIALS AND METHODS

The material comprised of first seed progenies (second somaclonal generation - SC_2) of 25 in vitro selected resistant barley plants (first somaclonal generation-SC_1) and one parent cultivar Dissa as control. The SC_1 plants were obtained by the procedure described earlier [1]. The experiment was conducted in glass house and field conditions.

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Glass house experiment: Progenies of 25 SC_1 lines obtained from 15 callus lines and Dissa were sown in pots (15 x 15 cm) replicated four times in a randomized fashion. 12 seeds/pot were sown and 7 seedlings/pot were retained as far as possible. Observations were recorded for seedling mutations and tiller number/plant. Cytological analysis was done on root tips taken out from some of the plants which showed variation. Root tips were fixed in aceto-alcohol (1:3) and preserved in 70% alcohol. 1% propionocarmine was used for staining.

Field experiment: Progenies of 19 SC_1 lines obtained from 13 callus lines and one parent cv. Dissa were sown in R.B.D. with three replications for SC_2 generation. Each plot consisted of a single row of one meter length. Data were recorded on three plants in each plot for plant height, spikelet number, kernel length and breadth, kernel length–breadth ratio, test weight and yield/plant. Disease reaction against *H. sativum* was recorded on 45-day-old plants after a week of inoculation with spore conc. of 60 conidia/µl. Scoring was done on a 0–9 scale.

RESULTS AND DISCUSSION

Two mutant seedlings obtained in glass house experiment were: i) internodeless (Int^-): three similar mutants characterized by thick, short and dark green leaves without any leaf sheath were obtained in line AT-28. These mutants did not have any internode growth and died before flowering (Fig. 1:1). ii) Freak narrow leaf (Fnl^-): this was obtained in line AT-42-2 characterized by freak narrow leaves with wrinkled light green leaf blade and died before flowering (Fig 1:2).

Cytological analysis of root tips showed multilobed nucleus in most of the cells of line AT-28 which showed Int^- mutant (Fig. 1:3). Bilobed nucleus was observed in somaclonal lines AT-37-3 and 3BT-1. All the plants showed normal diploid chromosome number (2n = 14). Relative stability of chromosome number among barley plants has been reported [2, 3].

Mean, range of variation, and phenotypic and genotypic coefficients of variation for the characters are shown in Table 1. All the characters showed significant increase over parent

Table 1. Mean and variation for morphological characters of SC_2 generation of barley

Parameter	Plant height (cm)	Spikelet numbers	Disease score	Test weight (g)	Kernel length (mm)	Kernel breadth (mm)	Kernel L/B ratio	Yield per plant (g)
Mean	69.4	85.3	4.0	36.2	10.0	3.3	3.0	16.1
Range	54.5–79.7	67.0–96.0	2.3–5.6	23.2–42.9	9.3–10.6	2.8–3.5	2.8–3.3	3.6–29.5
PCV	15.66	15.19	39.00	23.90	6.41	7.94	8.32	80.01
GCV	8.37	6.94	20.15	13.58	3.16	3.87	3.17	42.42

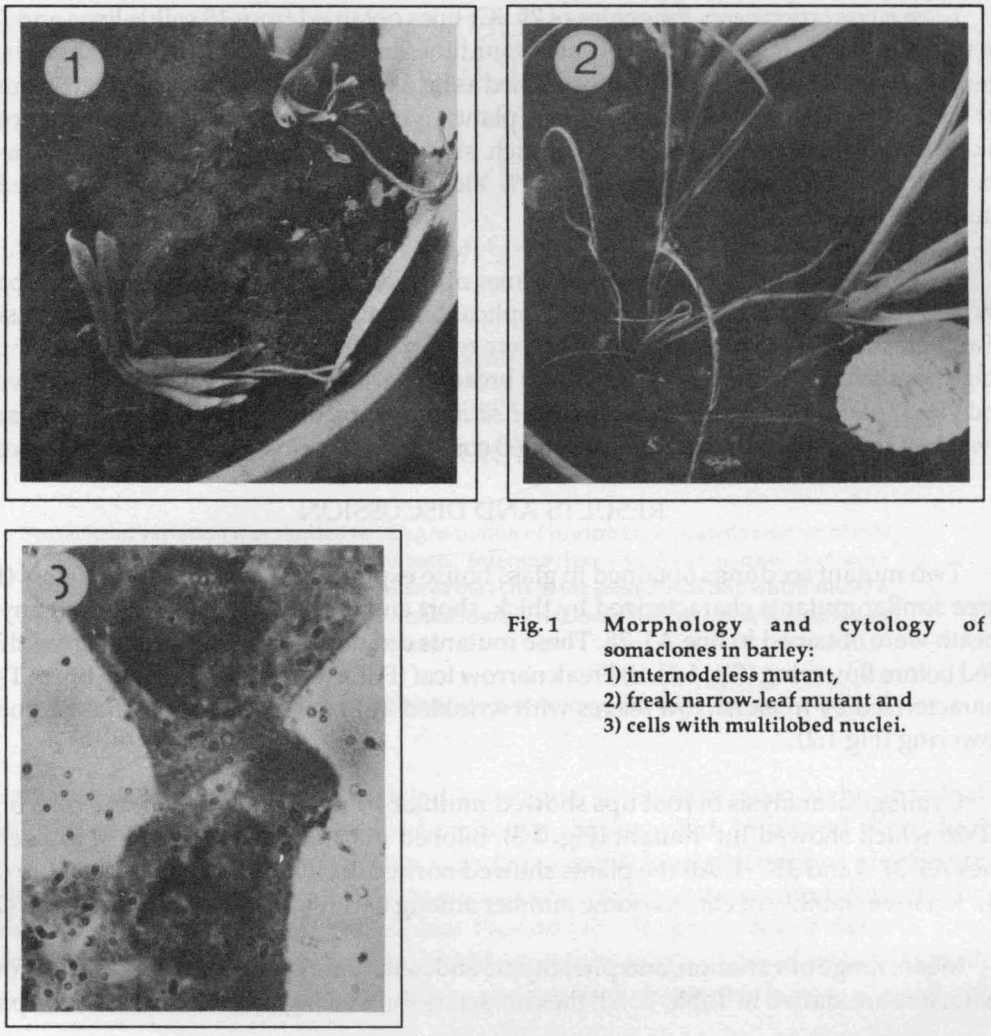

Fig. 1 Morphology and cytology of somaclones in barley:
1) internodeless mutant,
2) freak narrow-leaf mutant and
3) cells with multilobed nuclei.

cultivar in some somaclonal lines except for yield/plant where ten somaclonal lines showed yield at par with the donor parent. Plant height and test weight showed significant variation between somaclonal lines within a callus line. Significant variation for morphological characters in barley has been reported earlier [4, 5].

The original material for the study was obtained through in vitro selection and the disease resistance pattern of SC_1 plants was reported by Chawla and Wenzel [1]. In SC_2 generation 50% somaclonal lines showed same type of disease reaction as in SC_1 generation, while 15% of SC_2 lines showed conversion of resistance to intermediate type of reaction.

Remaining 35% of the lines showed breakdown of resistance. Arny [6] reported that resistance to *H. sativum* in barley is controlled by one factor pair susceptibility being dominant. Reversion of resistance of SC_1 lines to intermediate and susceptible types may be due to differences in pathogen strains used or the resistance in SC_1 lines was due to epigenetic causes or physiological changes rather than true genetic causes and was eliminated after one generation of sexual cycle.

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