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Variation of *Helminthosporium* resistance and biochemical and cytological characteristics in somaclonal generations of barley

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Abstract

SC_2 and SC_3 progenies of nineteen *in vitro* regenerated barley plants (SC_1) from resistant calli selected against purified culture filtrate of *Helminthosporium sativum* and one parent 'Dissa' genotype were studied for stability of resistance and protein, soluble protein, maltose and saccharose contents. Cytological studies were also carried out on the SC_3 generation. Stability of resistance to *Helminthosporium sativum* was found in 50% of the somaclonal lines. Significant variation among different somaclonal lines and among different callus lines from which the plants were regenerated were found for yield, disease score and biochemical characters assessed except saccharose content in the somaclonal lines. Significant increase and decrease over the donor parent for most of the characters were obtained. Cytological abnormalities such as multilobed nuclei, multinucleate cells, abnormal anaphase and mixoploidy were also observed.

Introduction

Exploitation of somaclonal variation with or without *in vitro* selection, has led to the isolation of disease resistant plants in various species of dicotyledonous plants and cereals (*cf.* Wenzel 1985 for review). Ideally a character that has been selected through tissue culture should be expressed at the plant level and stability inherited in subsequent generations. Plants with morphological and biochemical changes have also been isolated from somaclonal variation. Larkin *et al.* (1984) have made extensive studies of many morphological and biochemical traits in somaclones of wheat. Somaclonal variation for morphological traits has also been reported in cereals like rice (Oono 1986), maize (Edallo *et al.* 1981) and barley (Dunwell *et al.* 1986; Sozinov *et al.* 1988). In this experiment, seeds of self pollinated barley plants resistant to *H. sativum* toxins isolated through *in vitro* selection of calli against purified culture filtrate produced by *H. sativum* were taken.

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Studies were conducted to investigate stability of resistance and yield in these somaclonal second generation (SC_2) progenies. Biochemical characters like protein and saccharide contents were estimated in somaclonal third (SC_3) generation. The cytological basis of somaclonal variation was also studied in SC_3 generation.

Materials and methods

The somaclonal first (SC_1) generation: Callus culture from immature embryos and its regeneration procedure have been reported previously (Chawla and Wenzel 1987a). *In vitro* selection of callus against purified culture filtrate of *H. sativum*, regeneration of plants from resistant calli and *in vivo* testing of plants has also been described earlier (Chawla and Wenzel 1987b). Progenies of these 19 SC_1 regenerants comprising 9 resistant plants and 10 plants with intermediate disease reaction, and the parent cultivar 'Dissa' were sown in a randomised block design (RBD) with 3 replications. Each plot consisted of a single row of one metre length.

Assessment of variation in the SC_2 generation: a) Grain yield [g] was estimated after threshing. b) Disease reaction: *H. sativum* PK & B (strain No. 354.3) spore suspension with 60 conidia per mm^3 was sprayed on to plants after one and a half months of germination. Disease scoring was performed one week after inoculation on a 0-9 scale.

Assessment of variation in the SC_3 generation: a) Biochemical analysis: Total and soluble proteins, maltose (reducing saccharide) and saccharose (non-reducing saccharide) were estimated in near infra-red (NIR) analyser (*Inframatic 8180, Per Con Prüfgeräte,* Germany). 15-20 samples were analysed in the laboratory using standard procedure for calibration of the NIR analyser. The data were subjected to statistical analyses in RBD and nested design. b) Cytological studies: For chromosome counts, root tips of germinated SC_3 seeds were pretreated in distilled water at 1 °C 18 h followed by 0.1% colchicine treatment for 5-6 h. Root tips were fixed in 1:3 acetoalcohol for one day, hydrolysed in 1M HCl at 60 °C for 5 min and stained with 1% propionocarmine. For study of mitotic abnormalities, the above mentioned procedure except pretreatment with colchicine was followed.

Results and discussion

Different somaclonal lines showed significant mean squares in RBD analysis for disease score, yield/plant and all the biochemical characters except for saccharose content (Tables 1 and 2). Analysis in nested design showed that variation between callus lines was significant for all the characters including saccharose content.

Disease reaction recorded on *in vitro* regenerated plants of the SC_1 generation, as reported by Chawla and Wenzel (1987b), alongwith the responses to disease obtained in the SC_2 generation have been shown in Table 1. The mean disease score from each line ranged from 2.33 to 5.66. Out of 9 resistant SC_1 plants, 3 were resistant and 3 moderately resistant in the SC_2 generation, while 6 SC_2 lines out of 10 SC_1 plants which were intermediate in disease response again showed intermediate

type of resistance. Intermediate disease reaction means few brown spots and at a later stage of plant development. The remaing 7 somaclonal lines showed breakdown of resistance and were susceptible to *Helminthosporium* disease. Thus 50% of the SC_2 somaclonal lines

Table 1. Pattern of disease resistance against *Helminthosporium sativum* in SC_1 and SC_2 generations and cytological abnormalities in SC_3 generations of barley.

Somaclonal line	SC_1	SC_2		SC_3
	Disease reaction	Disease reaction	Mean disease score	Cytological abnormality
AT-1-1	R	R	2.33	N
AT-1-2	R	R~I	3.33	N
AT-25-1	R	R	3.00	N
AT-25-2	I	S	5.66	N
AT-25-3	I	I	3.66	N
AT-35-3	I	S	5.33	binucleate
AT-37-1	I	I	4.00	bilobed
AT-38	R	R~I	3.33	mixoploid
AT-40-2	R	R~I	3.33	N
AT-43-1	R	I	3.66	N
AT-43-2	R	I~S	4.33	N
AT-67-1	R	R	3.00	bi- and trilobed
AT-67-2	I	I	4.00	N
3BT-1	I	S	4.66	bilobed
3BT-2	I	I	4.00	binucleate
3BT-3	I	S	4.66	bi- and trilobed
T-1	I	I	3.66	N
T-43	R	S	5.00	N
T-125	I	I	3.66	multinucleate, multilobed
Dissa	S	S	5.33	N

Category of disease reaction on SC_2 lines: Resistant (R): DS≤3.00; Intermediate (I): DS 73.00 to 4.50; Susceptible (S): DS>4.50; N - Normal.

showed the same type of disease reaction as in the SC_1 generation. This indicates inheritance of the resistance trait. Reversion of resistance may be due to difference in pathogen strain used to test the disease response and secondly in the SC_1 and SC_2 generations the plants were tested at different stages. In SC_1 generation the plants were *in vitro* regenerated and screening for resistance was done at 2-3 leaf stage to know whether the toxin resistant callus transmits the resistance character at plant level or not. While in SC_2 generation the plants were field grown and testing was done from young to adult stages of plant and in natural conditions. In the SC_2 generation vatiation in resistance can be due to segregation of genes also. The complete breakdown of resistance in some lines perhaps indicate that resistance conferred in SC_1 plants was due to some epigenetic causes or physiological changes rather than true genetic causes.

Table 2. Mean and range of variation for yield in SC_2 and biochemical characters in SC_3 generations of barley.

S.l.No.	Somaclonal line	Yield [g plant^{-1}]	Protein [%]	Soluble protein [%]	Maltose [%]	Saccharose [%]
1	AT-1-1	9.63	13.42	3.11	1.36	2.88
2	AT-1-2	11.77	13.99	3.25	1.12	2.97
3	AT-25-1	22.21	12.81	3.09	0.97	2.64
4	AT-25-2	18.85	14.35	3.31	0.94	2.74
5	AT-25-3	16.94	14.95	3.21	1.00	2.74
6	AT-35-3	18.75	14.46	3.25	0.98	2.87
7	AT-37-1	13.71	15.07	3.27	0.74	2.94
8	AT-38	29.56	15.73	3.78	1.10	3.08
9	AT-40-2	29.50	15.22	3.44	0.84	3.00
10	AT-43-1	19.91	13.89	3.11	1.13	2.97
11	AT-43-2	9.91	15.64	3.52	0.89	3.13
12	AT-67-1	3.91	15.61	3.59	0.92	3.13
13	AT-67-2	3.66	15.11	3.53	1.08	3.05
14	3BT-1	12.39	14.28	3.16	0.99	3.15
15	3BT-2	15.27	12.71	2.81	1.07	3.26
16	3BT-3	20.92	12.25	2.64	1.18	3.20
17	T-1	16.83	14.13	2.91	1.03	3.10
18	T-43	6.08	12.82	2.91	1.23	3.21
19	T-125	18.46	12.84	2.84	1.22	3.13
20	Dissa	25.06	14.27	3.17	1.04	3.26
	Mean	16.16	14.17	3.20	1.04	3.20
	Range	3.66	12.25	2.64	0.74	2.64
		29.56	15.73	3.78	1.36	3.26
	C.D. at 5%	8.44	1.60	0.50	0.25	0.46

The mean and range of variation for various characters in SC_2 and SC_3 generation have been shown in Table 2. Significant differences in somaclonal lines from parent Dissa were obtained for higher soluble protein content in AT 1-1 while low for protein and soluble protein content in 3 BT-3 and low maltose in AT 37-1. None of the somaclonal lines showed significantly better yield as compared to parental line, however, significantly low yield was seen in large number of somaclonal lines. Variations have been observed for specific proteins in barley (Karp *et al.* 1987) and reducing saccharides in tobacco (Burk and Matzinger 1976).

Cytological analysis of somaclonal lines showed normal chromosome number, 2n = 14. However, mixoploidy was observed in somaclonal line AT 38 (Fig. 1). Besides chromosome number variation, mitotic abnormalities were also observed (Table 1). Multilobed nucleus (bi-or trilobed) characterised by almost round vacuolated structures (Fig. 2 and 3), multinucleate cells (Fig. 4) and abnormal anaphase showing cells with 6 and 8 chromosomes at two poles during mitosis (Fig. 5) were observed. The frequency of multilobed nuclei was more as compared to any other cytological abnormality. Enlarged and irregularly lobed nuclei with grouped clusters of upto 12 ovoid nuclear bodies in callus cultures of tobacco have been reported (Naylor *et al.* 1954). Since these bodies were found in tight groups it was impossible to determine

whether they were independent nuclei or distended lobes of enlarged interphase nucleus. Binucleate cells showed no cell plate formation indicating some changes perhaps at genic level have occurred which interferred in cell plate formation. These types of abnormal mitotic events may be due to selective inhibition on cell cycle stages by hormones (Nagl 1972) or genetic control of mitotic event (Hartwell *et al.* 1974) where mutations have led to different abnormalities.

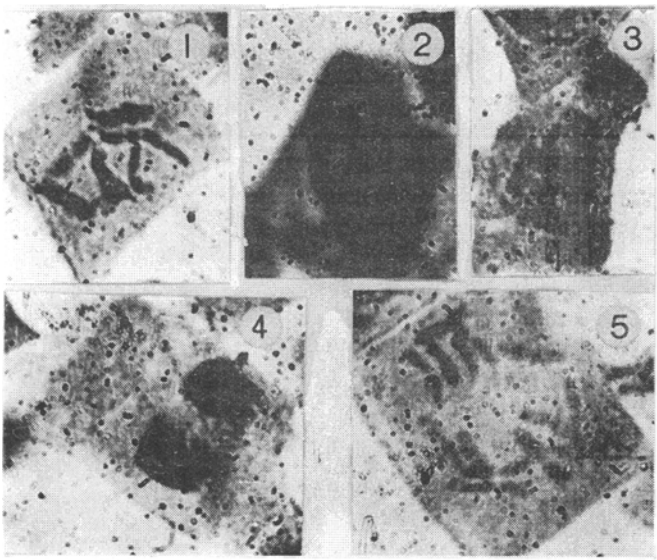

Fig. 1-5: Cytological abnormalities: 1 - Less chromosome number. 2 - Bilobed nucleus. 3 - Trilobed nucleus. 4 - Binucleate cell. 5 - Abnormal mitotic anaphase.

It may be concluded that *in vitro* selection against biotic stress has been steadily transmitted through sexual reproduction in 50 % of the lines of barley.

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