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PHENOTYPIC VARIATIONS AMONG PROGENY OF BASMATI SOMACLONES

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This study was undertaken on M_2 progeny of three rice (*Oryza sativa* L.) var. Basmati-370 *in vitro* regenerants named Bas-87-1, Bas-87-2, and Bas-87-3. Bas-370 was used as control. It was noted that the germination frequency in the progenies of all the three regenerants was lower in comparison with the control, being lowest in Bas-87-2. Visually, three types of chlorophyll variations i.e. albino (lacking chlorophyll), viridus (light green), and striated (whitish streaks) were observed. Some agronomic data is also reported in this paper which reveals that short statured Basmati lines resistant to lodging and high yielding can be selected through tissue culture.

Key words: Somaclonal variant, Basmati rice, Chloroplast.

INTRODUCTION

Basmati-370 is world's finest quality rice grown in Pakistan. However, being long statured it is highly susceptible to lodging resulting in decreased yields. Besides, disease susceptibility, low fertilizer response, etc. are also associated with this variety. The variety is pure breeding hence its germplasm has less variation. A multi-dimensional approach is essential to induce and conserve variation in this variety. Tissue culture is an effective tool in this regard [1]. The variation generated by tissue culture termed as somaclonal variation may result in both homozygous and heterozygous mutants [2]. They might also be aneuploids, mixoploids and chimeras [3]. Selection and purification through successive generations is necessary to evolve a true breeding line. In view of the extreme importance of this commodity, both at the national and international level there was a genuine need to explore non-conventional approaches to supplement the existing breeding programme. Tissue culture because of its proven success in many areas was the choice technology.

Many researchers [4,5] have reported a high frequency of chlorophyll mutants in plant tissue culture regenerants of rice, but no work has been reported on Basmati varieties. In the present study, the segregating pattern of different agronomic traits in M_2 progeny of three callus born regenerants of Bas-370 has been analysed and the results interpreted. Since, the callus cells under the influence of culture media, are mutated, we have supposed them to be M_0. While the plants formed out of these calli are M_1 and the first head progeny from the plants is taken as M_2.

MATERIALS AND METHODS

Basmati regenerants obtained from calli were transferred to soil media in standared size pots when they were 4 to 6 inches in length. Physical conditions of temperature and light were maintained as $28^{\circ} \pm 2^{\circ}$ and 16 hours day length with a light intensity of Ca 1000 lux for hardening. The regenerants labelled as Bas-87-1, Bas-87-2 and Bas-87-3 were raised to maturity in the glass house. Grains were harvested on June 4, 1987. Dormancy was broken by storing the grains at a temperature of $50^{\circ} \pm 2^{\circ}$ for five days. Before nursery raising in wooden trays, the seeds were sterilized with 5 % sodium hypochlorite for 15 minutes. The soil used was taken from rice fields mixed with farm yard manure in a ratio of 10 : 1. Sterilized, rinsed and pregerminated seeds were sown on these trays. The nursery was maintained in the glass house and the temperatures and photoperiods required for rice were adjusted in the glass house. Observations were recorded after 15 days during which period there was a visual leaf differentiation of striated, viridus and albinos in the progeny. These variants were grown to maturity in the glass house. Normal progeny, along with the control was transferred to field conditions at Kala Shah Kaku. Agronomic data was recorded on plant height, number of tillers per plant, number of productive tillers per plant, panicle number and panicle length. Mean values and standard deviations were calculated for all parameters noted.

RESULTS AND DISCUSSION

Data regarding germination frequency and chlorophyll mutations in M_2 progenies is reflected in Tables 1 and 2.

Low germination percentage can be attributed to deleterious mutations in the progeny or to seed dormancy. Although thermotherapy was done yet the conditions could have not standardized for mutant progenies prior to nursery raising.

Table 1. Germination frequency and chlorophyll mutations in M_2 progeny of three somaclones.

Plant type	No. of seeds sown	Germination (% age)	Chlorophyll mutants (% age in plantlets)
Bas 87-1	460	328 (71.3)	4 (1.2)
Bas 87-2	737	432 (58.6)	13 (3.3)
Bas 87-3	496	383 (77.2)	28 (7.31)
Total Data	1693	1143	45 (3.94)
Bas-370	250	225 (90)	(0)

Table 2. Nature and distribution of chlorophyll mutation among M_2 progeny of somaclones

Plant type	Viridus	Striated	Albino	Total (% age of chlorophyll mutants in three progenies)
Bas 87-1	4 (8.9)	–	–	4 (8.9)
Bas 87-2	–	3 (6.7)	10 (22.2)	13 (28.9)
Bas 87-3	1 (2.2)	9 (20.0)	18 (40.0)	28 (62.2)
Total	5 (11.1)	12 (26.7)	28 (62.2)	45 (100).

Three types of chlorophyll mutants were observed.

(i) Albinos which lacked the chlorophylls completely and could not survive (till maturity) indicating that they were true mutants.

(ii) Viridus which were light green.

(iii) Striated green plants with white strip.

Viridus and striated reverted to normal green plants turning at vegetative stage. Cell population was homogenously green in all these plants except one striated.

Chloroplasts have their own autonomously replicating DNA. The entire nucleotide sequence was recently achieved for the 121, 024-bp DNA molecule in liverwort chloroplast [6] and for the 155,844-bp molecule in tobacco chloroplast [7]. Contained within the molecule are genes for the four ribosomal RNAs (23 S, 16 S, 5 S and 4.5 S), 37 transfer RNA genes encoding 32 species (liverwort and tobacco differ in the number of such genes), and open reading frames for possibly 55 polypeptides ranging in length from 31 to 2136 amino acids. Many chloroplast encoded genes have post transcriptional and post translational regulations [8,9]. It behaves like different operons and is activated by physiological changes [10]. Chlorophyll variations in viridus and striated might be due to operon repression and might be linked with growth stages. The repressed genes could not show their function at emergance and seedling stage but were functional at vegetative stage due to some unknown favourable physiological changes. Chlorophyll is affected by 2,4-D [11]. One plant remained striated even to maturity. Chloroplast deformation may be due to the inherited 2,4-D or its amino acid conjugates [12,13], taken up from the medium by the seeds thus resulting in mutation of the genes responsible for chloroplast synthesis. Sun *et al.* (1979) reported, lack of synthesizing ability of fraction 1 protein in such plants [4]. The cooperation between the chloroplast and nuclear genomes has been extensively studied and is established. Thoroughly studied example is of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which catalyzes fixation of CO_2 in photosynthesis, is composed of two nonidentical subunits. The large subunit, RbcL (M_r. 55 Kd), is encoded by chloroplast DNA, the small subunit RbcS(M_r. 14-16.5 Kd, depending on the species), is encoded by nuclear DNA. Specific recessive nuclear mutant genes (plastome mutator genes), when in a homozygous condition, cause rather frequent plastid mutations. The plastid mutations subsequently are inherited in a non-Mendelian, uniparental pattern typical of organelle genes [14].

Data from plant height, total tillers, productive tillers, panicle length and peduncle length, number of plants, their means and standard deviation is given in Table 3. Mean plant height is lesser in M_2 of Basmati-87-1 and the standard deviation in all the three progenies is higher as compared to control. As a rule, higher the number of observations, lower should be the standard deviation. Lower standard deviation in the control is due to its homogenous nature. A short statured plant in such a progeny could thus be selected for further testing. Number of productive tillers and total tillers is higher in all the three progenies. Contribution of productive tillers is the highest in grain yield among all the yield components [15]. High number of total tillers, however can take up more nutrients and can lower the yield. Panicle length and peduncle length is also decreased in the progenies tested. This can cause a slight decrease in the yield but could be an effective criterian for selection against lodging. Variations in such parameters were also reported by other researchers [16,17,18].

Two rice plants with multiple panicles were selected from the chlorophyll mutants. Which indicates that the genes responsible for multiple panicles are either closely

Table 3. Agronomic data of somaclonal variants.

Plant type	No. of plants studied	Plant height	Total tiller	Productive tillers	Panicle length	Ped. length
Bas 87-1	14	*178.8 ± 86	39.5 ± 7.6	35.0 ± 8.1	27.3 ± 1.3	32.0 ± 2.2
Bas 87-2	35	177.3 ± 10.1	44.6 ± 12.8	35.8 ± 10.1	27.5 ± 1.6	32.6 ± 3.5
Bas 87-3	33	174.3 ± 10.6	46.7 ± 11.5	38.4 ± 10.8	27.0 ± 1.6	32.4 ± 3.8
Control	5	177.8 ± 6.8	26.6 ± 3.4	24.2 ± 2.9	31.4 ± 0.9	38.8 ± 1.9

*Mean ± SD.

associated with nuclear genes encoding plastid proteins or secondly they have some type of interaction. Third possibility may be that a wide spectrum of genes is mutated within single plant.

The study revealed that tissue culture technology can be successfully used to breed short statured Basmati-370 mutants which are both high yielding and resistant to lodging.

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