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IN VITRO TECHNIQUES AND MUTAGENESIS FOR THE GENETIC IMPROVEMENT OF POTATO CVS. DESIREE AND DIAMANT

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Abstract

The aim of the present study was to get somaclonal variants and induced mutants of potato for desirable characters with special emphasis on yield and yield components in two cultivars of potato viz., Desiree and Diamant. Inter-nodal explants from both the cultivars were incubated for 14-20wks in callus inducing medium comprising MS salts supplemented with NAA (1.0mg/l) and BAP (0.5mg/l) for obtaining somaclonal variants. For mutation induction, 10 week old, well proliferating calli was exposed to 5-50Gy of gamma irradiation. The regenerated plants were analyzed on the basis of morphological characters for variation. The plants were screened on the basis of average shoot height, number of shoots, number of nodes/shoots, average tuber number, tuber size, tuber weight and number of eyes / tuber**.** Three variant lines (SV1, SV2 and SV3) and 6 gamma mutant lines (GM1, GM2, GM3, GM4 GM5 and GM6) were selected on the basis of better yield and other agronomic characters. The current study demonstrates the production of useful variants both by tissue culture and gamma irradiation in potato.

Introduction

Potato (*Solanum tuberosum* L.) is an economically important vegetable crop of Pakistan. It is the fourth most important crop by volume of production; it is high yielding, having a high nutritive value and gives high returns to farmers. Pakistan is the seventh largest potato producing country in the world. Potato was cultivated over an area of 248.59 thousand hectares, with a production of 2819.21 thousand tons during 2007-08 fiscal year (Minfal, 2008). Its high nutritional value and broad adoptability nature increases its consumption and demand day by day. It is propagated predominantly by asexual means (tubers) and propagation by true seed is primarily used for breeding purposes.

The recent large increase in acreage was reached by an intensification of the cultivation in existing potato growing areas, as well as by introduction of the crop in new areas and to inexperienced farmers. Hence, many problems, like diseases and pests, became more hazardous and a large number of farmers are lacking knowledge of the right cultivation technique. Lack of availability of sufficient quantities of good seed and low purchasing power of the farmers, forces them to rely on seed sources of doubtful quality or own production, for which most of them do not have the proper skills.

In recent years, tissue culture technology has provided a tool to complement conventional plant breeding. Plant tissue culture is recognized as a source to generate useful genetic variability (somaclonal variation) for crop improvement (Larkin & Scowcroft, 1981, Karp & Bright, 1985, Evans *et al*., 1989, Brar & Jain, 1998). The generation of somaclonal variation has been applied in crop improvement with the intention of including and exploiting useful and economically valuable characters that may not be readily available within other sources of germplasm (Juned *et al*., 1991). In several commercial varieties of potato, tissue culture induced variations were observed in

a wide range of characters, such as plant morphology, tuber characteristics, disease resistance, isoenzymatic pattern, tuber proteins and chromosome number and structure (Jelenic *et al*., 2001, Thieme *et al*., 2005). Deverno (1995) reported that the frequency of somaclonal variation increased with the duration of *In vitro* culture, either as callus or cell suspension.

Another source of generating variations in plants is by mutagenesis (physical and chemical). Radiation induced mutations and chemical mutagenesis have been extensively used for the improvement of crop plants (Shah *et al*., 2008). The combination of mutagenesis with *In vitro* techniques offers an efficient method for improving vegetatively propagated plants. These techniques allow induction of variation, selection and multiplication of the desired genotypes in a much shorter duration and smaller space than conventional methods.

The purpose of the present work was to select plants regenerated from calli of different origins (somaclonal variants or induced mutants) of potato cvs. Desiree and Diamant, having improved characters with special reference to yield potential and agronomic characteristics.

Materials and Methods

Callogenesis and plant regeneration: Callus was initiated from inter-nodal explants of potato (cvs. Desiree and Diamant) in MS medium supplemented with NAA (1.0mg/l) and BAP (0.5mg/l). For obtaining somaclonal variants, well-proliferated calli was incubated for 14 to 20 weeks in the same medium. For plant regeneration, the calli were transferred to regeneration medium-comprising MS salts supplemented with NAA (0.5mg/l) and BAP (2.0mg/l).

Gamma irradiation: Ten weeks old, well proliferating calli of both the cultivars with an average weight of 1.35gm was used for irradiation at different exposures of γ-rays (5, 10, 15, 20, 25, 30, 40 and 50Gy). After irradiation the cultures were shifted immediately on fresh hormone free MS medium, incubated for 5 days and then sub-cultured on callus inducing medium for further proliferation of callus. After 2 weeks, the calli were transferred to regeneration medium. The plantlets thus formed were used to get R1 and R2 generation for further analysis.

Selection of somaclonal variants and induced mutants: The selection of variants/mutants was based on the following morphological characteristics: stem height, number of nodes per shoot, average tuber number, average tuber size, average tuber weight, number of eyes and number of shoots per plant.

Statistical analysis: Following the analysis of variance (ANOVA), means were used to find simple correlations between the performance of genotypes in various *In vitro* treatments and the corresponding performances of these genotypes in *In vivo* conditions for various characters. Duncan multiple range test was also used where applicable. (Steel & Torrie, 1980.).

Results

Morphological characters of selected variants of cvs. Desiree and Diamant: The plants regenerated from long-term incubated and well proliferated calli of both the cultivars were hardened in the glasshouse and shifted to tunnels to obtain R1 and R2 generations. In

tunnels the plants were screened on the basis of their morphological characters and a total of 7 variants were selected, 3 from cv. Desiree and 4 from cv. Diamant.

All the selected somaclones of cv. Desiree showed an increase in height (except SV2), number of nodes/shoot, number of shoots and average tuber number as compared to control. An increase in average tuber weight and number of eyes per tuber were observed in two variants (SV2 and SV3), while no significant difference was observed in average tuber size as compared to control (Table 1).

In case of cv. Diamant, an increase in the number of shoots and average tuber number was observed in all the selected variants as compared to control. An increase in plant height (SV4, SV7), number of nodes per shoot (SV5, SV6) average tuber size (SV4, SV5), average tuber weight (SV6, SV7) and number of eyes was observed in different variants as compared to control (Table 2).

Effect of different exposures of gamma- irradiation on callus proliferation: After 2 weeks of irradiation, an increase in the weight of callus was observed at the radiation doses up to 20Gy in both the cultivars as compared to control. In the present study, it was also observed that with further increase in the dose from 25 to 50Gy, there was a decrease in callus weight accompanied by necrosis (Fig. 1).

Morphological characters of selected mutants of cvs. Desiree and Diamant: The plants regenerated after mutagenic treatments with γ-rays of both the cultivars were shifted to glasshouse for hardening and then to field under controlled environment to obtain R1 and R2 generations. Mutants finally selected after R2 generation were analyzed on the basis of their morphological characters and 11 plants (6 from cv. Desiree and 5 from cv. Diamant) were finally selected. Statistical analysis of the individual characters supported the existence of significant variation observed among the different mutants.

In both the cultivars, Desiree and Diamant, most of the mutants showed an increase in plant height, number of nodes /shoot, number of shoots and number of eyes per tuber as compared to control. Almost all the mutants showed an increase in the average number of tubers, tuber size and tuber weight in comparison with non-treated control in both the cases (Table 3 and 4). All the selected variants and mutants showed an increase in the number of tubers as compared to control in both the cultivars.

Discussion

Somaclonal variations are considered to be a good supplement to conventional crop improvement. There is evidence in different crops that the variant characteristics obtained from culture of somatic tissues, is transmitted successfully to the progeny in terms of these desirable characteristics. Another source of creating variations in plants is by induced mutations. Induced mutation techniques have been successfully used to improve yield, quality, disease and pest resistance in crops, or to increase the attractiveness of flowers and ornamental plants (Brar & Jain, 1998).

A combination of *In vitro* technology and radiation/chemical-induced mutagenesis has been recommended to improve cultivars of vegetatively propagated crops (Novak, 1991; Maluszynski *et al*., 1995). The use of *In vitro* cultures in mutation breeding offers several advantages over the *In vivo* techniques including, obtaining explants from pre-existing cultures and recovering mutants and rapidly micro propagating them under controlled environmental conditions.

Table 1. Characteristics of plants regenerated from callus tissues of *S. tuberosum* cv. Desiree.

No.	Height (cm)	No. of nodes/ shoot	Average tuber no.	Average tuber size (cm)	Average tuber weight (gm)	No of eyes	No. of shoots
Cont	72.2 ± 0.334^{c}	18.0 ± 0.282^{d}	8.4 ± 0.456^{c}	7.0 ± 0.282^{ab}	115.6 ± 0.219^{c}	8.4 ± 0.219^{c}	8.0 ± 0.282^{c}
SV1	77.8 ± 0.334^{a}	20.1 ± 0.224^{b}	$10.2 \pm .593^{b}$	6.4 ± 0.178^{b}	113.6 ± 0.357^{d}	8.0 ± 0.282^{c}	9.0 ± 0.282^{bc}
SV2	69.8 ± 0.521^{d}	19.2 ± 0.521^{c}	$10.8 \pm .334^{ab}$	7.2 ± 0.334^{a}	120.6 ± 0.456^{a}	11.2 ± 0.334^{a}	9.6 ± 0.4^{ab}
SV3	76.0 ± 0.748^{b}	23.0 ± 0.282^{a}	$11.8 \pm .334^{a}$	6.8 ± 0.219^{ab}	116.8 ± 0.334^{b}	9.4 ± 0.219^{b}	10.2 ± 0.334^{a}
$LSD_{0.05}$	1.722	1.24	1.484	0.874	1.180	0.899	1.059

Means followed by different letters in the same column differ significantly at p = 0.05 according to Duncan's new multiple range test.

Table 2. Characteristics of plants regenerated from callus tissues of *S. tuberosum* cv Diamant.

No.	Height (cm)	No. of nodes/ shoot	Average tuber no.	Average tuber size (cm)	Average tuber weight (gm)	No of eyes	No. of shoots
Cont	67.4 ± 0.334^{c}	17.4 ± 0.357^{c}	$8.0 \pm 0.219c$	6.8 ± 0.334^{ab}	116.2 ± 0.334^{c}	8.8 ± 0.334^{b}	7.2 ± 0.178^{d}
SV4	71.8 ± 0.438^{b}	11.2 ± 0.340^{e}	11.2 ± 0.334^{b}	7.0 ± 0.282^{a}	$118.0 \pm 0.632b$	11.2 ± 0.334^{a}	10.2 ± 0.593^{ab}
SV5	61.4 ± 0.357^{f}	20.6 ± 0.219^{a}	13.0 ± 0.632^{a}	$7.2 \pm 0.178a$	120.2 ± 0.521^{a}	11.3 ± 0.334^{a}	9.0 ± 0.282^{bc}
SV6	54.6 ± 0.456^{e}	19.4 ± 0.456^{b}	9.4 ± 0.219^{c}	5.9 ± 0.226^{b}	113.2 ± 0.521^{d}	7.2 ± 0.178^{c}	8.8 ± 0.334^{c}
SV7	75.0 ± 0.632^{a}	12.6 ± 0.219^{d}	10.2 ± 0.521^{b}	6.0 ± 0.282^{b}	115.0 ± 0.282^{c}	9.4 ± 0.219^{b}	11.2 ± 0.334^{a}
$LSD_{0.05}$	1.504	1.08	1.384	0.914	1.572	0.951	1.223

Means followed by different letters in the same column differ significantly at p=0.05 according to Duncan's New Multiple Range Test.

Table 3. Characteristics of plants regenerated from gamma-irradiated calli of *S. tuberosum* cv Desiree.

Plant no.	Height (cm)	No. of nodes/ shoot	Average tuber no.	Average tuber size (cm)	Average tuber weight (gm)	No of eyes	No. of shoots
Cont	72.2 ± 0.769^{c}	22.6 ± 0.606^{c}	10.2 ± 0.334^{e}	6.8 ± 0.334^{c}	108.2 ± 0.521^{d}	11.4 ± 0.357^{bc}	10.2 ± 0.334^{de}
GM1	79.2 ± 0.334^{a}	27.8 ± 0.348^{a}	18.8 ± 0.438^{a}	8.2 ± 0.334^{a}	118.2 ± 0.593^{a}	13.8 ± 0.334^{a}	15.4 ± 0.219^{a}
GM2	76.0 ± 0.748^{b}	27.8 ± 0.334^{a}	16.2 ± 0.178^{b}	8.0 ± 0.282^{ab}	118.4 ± 0.456^{a}	12.4 ± 0.219^{b}	14.0 ± 0.282^{b}
GM3	70.4 ± 0.456c	20.0 ± 0.489d	11.0 ± 0.489de	6.46 ± 0.166c	108.1 ± 0.606d	12.0 ± 0.282bc	11.0 ±0.40de
GM4	74.8 ± 0.867^{b}	21.2 ± 0.334^{d}	12.0 ± 0.282^{cd}	7.4 ± 0.219^{abc}	109.8 ± 0.334^{d}	12.0 ± 0.282^{bc}	11.2 ± 0.334^{d}
GM5	76.4 ± 0.456^{b}	24.4 ± 0.210^{b}	13.0 ± 0.282^{c}	7.2 ± 0.178^{bc}	115.2 ± 0.867^{b}	12.2 ± 0.438^{bc}	12.4 ± 0.456^{c}
GM6	70.4 ± 0.606^{c}	20.0 ± 0.40^{d}	12.8 ± 0.334^{c}	7.0 ± 0.282^{c}	112.0 ± 0.632^{c}	11.0 ± 0.40^{c}	10.0 ± 0.282^{e}
$LSD_{0.05}$	2.048	1.359	1.127	0.876	1.922	1.095	1.095

Means followed by different letters in the same column differ significantly at p= 0.05 according to Duncan's New Multiple Range Test.

Table 4. Characteristics of plants regenerated from gamma-irradiated calli of *S. tuberosum* cv Diamant.

Plant no.	Height (cm)	No. of nodes/ shoot	Average tuber no.	Average tuber size (cm)	Average tuber weight (gm)	No of eyes	No. of shoots
Cont	70.2 ± 0.593^{bc}	21.2 ± 0.334^{c}	11.6 ± 0.357^{c}	6.8 ± 0.093^{bc}	107.0 ± 0.489^{d}	13.0 ± 0.282^{b}	10.2 ± 0.334^{c}
GM7	69.0 ± 0.849^{c}	21.6 ± 0.726^{c}	12.2 ± 0.334^{c}	6.5 ± 0.089^{c}	107.4 ± 0.219^{d}	12.0 ± 0.282^{bc}	10.8 ± 0.334^{c}
GM8	75.0 ± 0.282^{a}	24.0 ± 0.282^{b}	16.8 ± 0.521^{a}	7.5 ± 0.097^{a}	116.0 ± 0.282^{a}	13.0 ± 0.565^{b}	14.4 ± 0.456^{a}
GM9	70.8 ± 0.657^{b}	19.4 ± 0.456^{d}	13.0 ± 0.282^{bc}	6.8 ± 0.101^{bc}	110.0 ± 0.282^{c}	12.0 ± 0.489^{bc}	11.2 ± 0.334^{c}
GM10	75.0 ± 0.632^{a}	25.8 ± 0.334^{a}	16.0 ± 0.489^{a}	7.6 ± 0.116^{a}	115.2 ± 0.769^{a}	14.4 ± 0.219^{a}	14.4 ± 0.219^{a}
GM11	73.4 ± 0.456^{a}	24.4 ± 0456^{ab}	14.2 ± 0.438^{b}	7.04 ± 0.046^{b}	112.2 ± 0.521^{b}	11.2 ± 0.334^{c}	13.0 ± 0.632^{b}
$LSD_{0.05}$	1.702	1.488	1.348	0.305	1.526	1.249	1.327

Means followed by different letters in the same column differ significantly at p=0.05 according to Duncan's New Multiple Range Test.

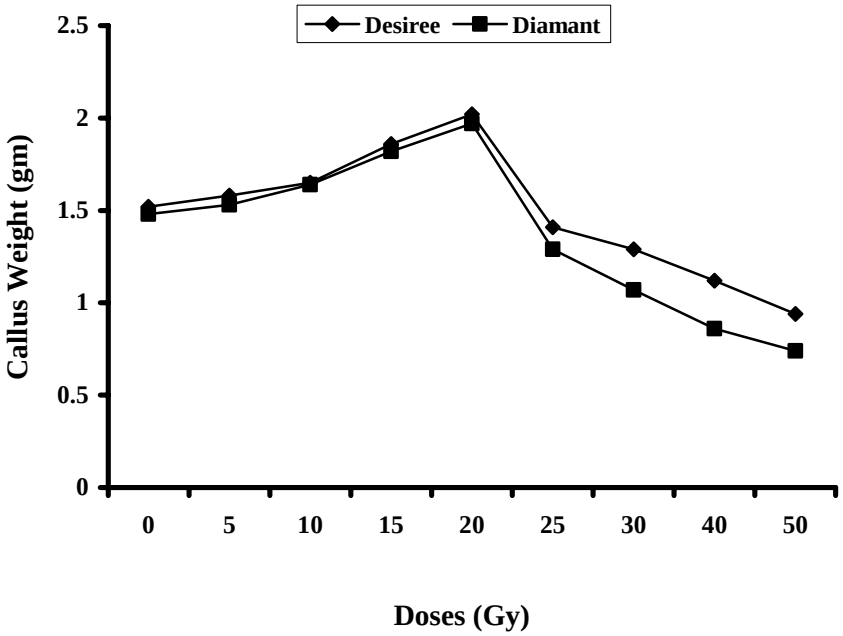

Fig. 1. Effect of gamma rays on callus proliferation in cvs. Desiree and Diamant.

In the present study, for the induction of somaclonal variants, internode-derived callus cultures were used instead of differentiated explants (apical or nodal meristem). This is because variations occur frequently in dedifferentiated cultures (callus and suspension cultures) and are less common in cultures where organized plant structures, such as preexisting meristems were maintained (Anthony, 1999). According to Babu *et al*., (1992), Ahuja (1998) and Larkin (1998), when already differentiated tissues are used for plant regeneration, there may be an increased rate of somaclonal variation through an intermediary callus phase. This source of variation could be exploited for crop improvement especially when other conventional methods have shown to be ineffective (Silvarolla, 1992).

Stress exerted by *In vitro* culture conditions may be an additional factor that increases mutation frequencies through impaired replication and drastic modifications of cell metabolism. McClintock (1984) suggested that the induction of genetic variability or any source of genetic instability during stress might be a prerequisite for adaptation.

The frequency of somaclonal variation has been reported to increase with duration of *In vitro* cultures either as callus or cell suspension (Deverno, 1995). In the present study, incubation period of callus was also prolonged and it was observed that that the duration of callus cultures has a marked effect on frequency of somaclonal variants. The extent and frequency of somaclonal variation is strongly affected by genotype.

In the present work, of the two cultivars used, it was observed that the number of regenerated variants in cultivar Desiree were more as compared to the cultivar Diamant. This may be due the difference in genetic make up of the two cultivars. Similar results were also reported by Gunn & Shepard (1981), who observed different number of variants in the regenerated plants of distinctive cultivars of potato, grown under identical conditions.

Somaclonal variation for plant height, number of leaves/plant, number of tubers/plant and tuber weight/plant in different potato cultivars has been reported by many workers (Berljak 1991., Alphonse *et al*., 1999, Jelenic *et al*., 2001, Yildirim *et al*., 2003, Bordallo *et al*., 2004). In the present study, variations were observed in plant height, number of shoots, number of nodes/shoot, average tuber number, tuber size, tuber weight and number of eyes as compared to control.

For mutation induction, the calli were irradiated with different doses of gamma irradiation (5-50Gy). It was observed that the callus weight increased up to the dose of 20Gy, and on further increase to 50Gy, a decline was shown in the weight of callus.

The increase in the weight of callus at low doses of gamma rays has also been reported by many workers in bean, orange and carrot (Bajaj, 1973; Spiegel-Roy & Koghba, 1973; Al-Safadi & Simon, 1990, 1995). A reduction in callus fresh weight at high doses as a result of gamma irradiation was also reported by Bajaj, (1973) and Omar (1988). This reduction in fresh weight may be caused by the reduced amount of endogenous growth regulators especially the cytokines, as a result of break down, or lack of synthesis due to irradiation.

Irradiation studies in potato have been found to produce different morphological characteristics including shallow eye, changed tuber shape, skin colour and tuber size (Ahloowalia, 1990, Arslanoglu & Atakisi , 1997, Al-Safadi *et al*., 2000,). Low doses of gamma irradiation can induce physiological and biochemical changes (Berezina & Kaushanskii, 1989), resulting in faster vegetative growth and early flowering (Al-Oudat, 1990). Castillo *et al*., (1997) suggested that doses for production of mutant in programs of plant improvement should not exceed 25-35Gy.

In the present study, an increase in the number of plants regenerated from irradiated calli was observed as compared to control up to the dose of 20Gy. Similar findings regarding the increase in the number of plants regenerated from irradiated callus by low doses of gamma irradiation has also been reported by Sidark & Suess, (1973); Kuzin *et al*., (1986); Venkatachalam *et al*., (1999); Musoke *et al*., (1999); Das, (2000); Karmarkar, (2001) and Kulkarni (2004); Singh (2009).

Both somaclonal variation and induced mutations result in the production of new genotypes with a limited change in the original genome. As a source of variation, somaclonal variation mimics induced mutation. Both somaclonal variation and conventional mutagenesis are complementary to and not a replacement for conventional plant breeding.

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