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Short Communication

Isolation of Somaclonal Variants for Morphological and Biochemical Traits in *Curcuma longa* (Turmeric)

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Five types of somaclonal variants were isolated through callus phase of vegetative bud among the 105 regenerants, based on the morphological traits at the culture conditions. The variants showing higher values of the metric traits than the regenerants and control with regard to morphological parameters in the first generation were selected for further evaluation in the second generation (V_2). The variants isolated based on the morphological traits were subjected to biochemical analysis such as curcumin, oleoresin and volatile oil contents and compared with the normal regenerants and the control plant. Significantly high curcumin, oleoresin and volatile oil contents (%) were observed in somaclonal variants when compared to the normal regenerants and also control plant. Somaclonal variation in turmeric is a new prospective for the genetic improvement of turmeric varieties.

Keywords: Somaclonal variants, turmeric, vegetative bud, callus

Turmeric (*Curcuma longa* L.) is one of the most important ancient spices of India and a customary item for export. Turmeric is known as the "Golden Spice" as well as the "Spice of Life". It has been used in India as a medicinal plant, and held sacred from time immemorial (Duke, 2007) and is reported to be a therapeutic agent for several major human diseases (HungHsu and Lii Cheng, 2007). The primary biological active constituent of turmeric is the curcumin, a polyphenol that has potent anti-inflammatory and anti-oxidant properties (Singletary, 2010).

Heritable genetic variation found in plants regenerated from any type of *in vitro* culture is termed somaclonal variation (Larkin and Scowcroft, 1981). In most cases, *in vitro* differentiation is a major cause of genetic variation (Swartz, 1991). Only random variations found in regenerated plants that are transmitted to the progeny through meiosis and are not reversible can be called as somaclonal variation (De Klerk, 1990). Such variation in callus regenerated plants has been documented in many plant species for a wide array of characters (Larkin and Scowcroft, 1981; Reisch, 1983; Vasil, 1986; Bajaj, 1990 and Karp, 1995).

Somaclonal variations offer a new source of genetic variability which can be exploited effectively in breeding programs designed to select the desirable characters in the improvement of economically important plants. In turmeric, natural genetic variation

is less due to vegetative propagation and lack of sexual cycle. Hence, the present investigation aims at the isolation of high yielding somaclonal variants through callus phase in turmeric variety Suguna.

MATERIAL AND METHODS

Turmeric variety Suguna, a genetically identical clone was used as a source material. The non-embryogenic vegetative propagule vegetative bud explants were sterilized and inoculated on Linsmaier and Skoog's Basal Medium (LSBM) supplemented with 2,4-D (3 mgl^{-1}) for induction of callus. The actively growing mass of callus was subcultured on fresh medium. The callus was cut into 0.5 cm^2 pieces and cultured on LSBM fortified with BAP (3.5 mgl^{-1}) for differentiation and regeneration. The cultures were maintained at a temperature of 25 ± 2^0 C with white fluorescent light at a photon density of 30-50 $\mu Em^{-2}\ s^{-1}$ under a photoperiodic regime of 16 hours light and 8 hours dark cycles.

Isolation of somaclonal variants:

The *in vitro* raised plants were screened at culture conditions to isolate somaclones from the regenerants based on the morphological variations and were named as 'Somaclonal Variants'. The variants and the normal regenerants were hardened using a potting mixture consisting of peat: perlite: vermiculate 1:1:1 (v/v) and maintained in hardening chamber under controlled conditions. The hardened and acclimatized plants were successfully transferred to field and their survival frequency was recorded.

Evaluation of somaclones based on morphological and biochemical traits:

The somaclones isolated were hardened and transferred to the field to study their morphological traits (plant height, number of tillers per clump, number of leaves per clump, leaf size, yield of rhizomes per clump and dry recovery) as V_1 generation (first generation following the *in vitro* phase) and compared with the normal regenerants and control (variety Suguna). The somaclones showing higher values of the metric traits than the regenerants and control with regard to morphological characters in the first generation (V_1) were selected for further evaluation in the second generation (V_2) through conventional vegetative multiplication. The somaclones and regenerants of V_1 and V_2 generations were analyzed for morphological traits and biochemical attributes like curcumin (ASTA, 1958) oleoresin (EOA, 1967) and volatile oil (ASTA, 1968) contents and compared with the control plant Suguna.

Statistical analysis:

The data obtained in the present study were analyzed statistically by one-way analysis of variance (ANOVA) to determine the variation between the treatments and Least Significant Difference (LSD) between any of the two means at $p = 0.05$ (the level of probability chosen for the t value) was determined.

RESULTS

Five different types of variants such as 'Narrow elongated leaf with thick short pseudostem' (SC1), 'Broad elongated leaf with very short pseudostem'(SC2), 'Broad elongated leaf with thick short pseudostem'(SC3), 'Broad short leaf with very short pseudostem' (SC4) and 'Broad short leaf with normal pseudostem' (SC5) were isolated through callus phase of vegetative bud among the 105 regenerants based on the morphological characters at the culture conditions. These variants were hardened and transferred to the field with 94 % survival frequency.

Somaclones isolated based on the morphological parameters (V_1 - V_2 generations) were subjected to biochemical analysis such as curcumin, oleoresin and volatile oil contents and compared with the normal regenerants and the control plant. The somaclone (SC1) was found to be superior with regard to plant height (110.42 cm) and rhizome yield (538.87 g) (Table 1). Further, it was found to be superior with regard to biochemical traits, with high curcumin (5.48%), oleoresin (15.23%) and volatile oil (7.16%) contents (Table 2). The somaclone "Narrow elongated leaf with thick short pseudostem" (SC1) was found to be superior when compared to other somaclones, normal regenerants and the control plant. It was observed that there exist highly significant differences with regard to morphological and biochemical traits among the somaclones.

Table 1. *Curcuma longa* L. variety Suguna : Morphological traits of variants, regenerants and control

Group	Type of Plants	Plant height (cm) M ± SD	No. of tillers/ clump M ± SD	No. of leaves/clump M ± SD	Leaf length (cm) M ± SD	Leaf breadth (cm) M ± SD	Yield of rhizomes / clump (g) M ± SD	Dry recovery (Mother + Pri.+ Sec. Rhizomes) M ± SD
Control Suguna	Normal plant	106.98 ± 0.16	1.91 ± 0.21	12.33 ± 0.14	45.89 ± 0.12	12.33 ± 0.09	528.89 ± 0.19	20.43 □ 0.09
Indirect Regeneration of Vegetative bud	Regenerants	107.16 ± 0.15	1.96 ± 0.13	12.41 ± 0.22	46.17 ± 0.16	12.41 ± 0.18	529.08 ± 0.07	20.81 ± 0.01
Somaclonal Variants	SC1	110.42 ± 0.44	2.94 ± 0.03	15.40 ± 0.03	48.77 ± 0.05	14.64 ± 0.04	538.87 ± 0.66	22.32 ± 0.04
	SC2	109.14 ± 0.15	2.54 ± 0.02	13.61 ± 0.03	47.19 ± 0.03	14.07 ± 0.07	534.67 ± 0.26	21.89 ± 0.04
	SC3	110.12 ± 0.38	2.90 ± 0.03	14.47 ± 0.04	48.09 ± 0.06	14.62 ± 0.03	538.78 ± 0.24	22.16 ± 0.06
	SC4	109.66 ± 0.25	2.55 ± 0.03	15.19 ± 0.01	46.86 ± 0.06	14.47 ± 0.05	535.65 ± 0.75	20.98 ± 0.03
	SC5	109.76 ± 0.18	2.43 ± 0.01	14.76 ± 0.03	48.16 ± 0.06	14.57 ± 0.05	537.82 ± 0.02	21.91 ± 0.08
	5% LSD	0.41	0.06	0.03	0.07	0.06	1.54	0.07

M– Mean of 10 replications; SD – Standard Deviation; LSD – Least Significant Difference

Table 2 *Curcuma longa* L. variety Suguna : Biochemical traits of variants, regenerants and control

Group	Type of Plants	Curcumin (%) M ± SD	Oleoresin (%) M ± SD	Volatile oil (%) M ± SD
Control Suguna	Normal plant	4.92 ± 0.17	13.51 ± 0.13	6.08 ± 0.14
Indirect Regeneration of Vegetative bud	Regenerants	5.03 ± 0.18	13.61 ± 0.17	6.13 ± 0.19
Somaclonal Variants	SC1	5.48 ± 0.02	15.23 ± 0.02	7.16 ± 0.03
	SC2	5.44 ± 0.03	15.18 ± 0.02	7.13 ± 0.05
	SC3	5.37 ± 0.02	15.16 ± 0.03	6.68 ± 0.05
	SC4	5.13 ± 0.03	14.32 ± 0.05	6.85 ± 0.03
	SC5	5.11 ± 0.03	15.11 ± 0.06	7.02 ± 0.03
	5% LSD	0.03	0.05	0.04

M – Mean of 10 replications ; SD – Standard Deviation; LSD – Least Significant Difference

DISCUSSION

In the present investigation it was possible to isolate somaclonal variants through callus phase of vegetative bud based on the morphological and biochemical traits in turmeric. The origin of somaclonal variation may be due to periodic subculturing of callus over an extended period of time, which undergo morphological and genetic changes such as polyploidy, aneuploidy, chromosomal aberrations, point mutation, alteration of methylation patterns or DNA amounts, selective sequence amplification or deamplification, tissue culture induced transposition activity, modification of organellar genome, transposable elements, genetic status of the donor plant, age of the explant, nutrient media, phytohormones, other extrinsic culture conditions as suggested by Nagl (1972), Cullis (1983), Day and Ellis (1984), D' Amato (1985), Ball and Seilleur (1986), Brettel *et al.* (1986), Bajaj (1990), Kaepller *et al.* (2000), Jain (2001) and Anjanasree *et al.* (2012).

The plants showing higher values of the metric traits than the parental type with regard to morphological characters (such as plant height, number of tillers per clump, number of leaves per clump, leaf size, yield of rhizomes per clump and dry recovery) in the first generation (V_1) were selected for further evaluation in the second generation (V_2). In the present study, five somaclones isolated based on the morphological parameters from indirect regeneration of *in vivo* vegetative bud (V_1 – V_2 generations) were subjected to biochemical analysis such as curcumin, oleoresin and volatile oil contents and compared with the normal regenerants and the control plant. Similar results have been reported by Mathur *et al.* (1989) in aromatic crops and Ravindra *et al.* (2004) in rose-scented geranium. Further, the present findings coincides with the reports of Bajaj (1986) and Bajaj *et al.* (1986) who have observed range of morphological variations in Cereals and Grasses.

'Somaclone' refers to the individual variant regenerated *in vitro*. Somaclonal variation is an expression of plant cell culture system that involves a stage of disorganized cell growth or adventitious meristems. Genetic variations occur in undifferentiated cells, isolated protoplasts, calli and tissue, which are manifested as morphological traits of regenerated plants (Batra, 2001). Novel variants have been reported among somaclones. Somaclonal lines may be more variable than breeder lines for most agronomic yield components and quality characters (Hanson *et al.*, 1994).

Further, callus-derived somaclonal variation based on morphological and biochemical parameters have been reported by Popescu *et al.*, (1997) in strawberry, Pajević *et al.* (2004) in sunflower, Anu *et al.* (2004) in *Capsicum annuum,* Jibu *et al.* (2006) in tea, Shen *et al.* (2007) in *Dieffenbachia,* Rajeswari *et al.* (2009) in Sugarcane, Shah *et al.* (2009) in wheat, Park *et al.* (2010) in Rice, Thepsithar *et al.* (2010) in *Caladium*, Li & Bruneau (2010) in St. Augustinegrass, Yari and Farahani (2011) in Olive, Yari *et al.* (2011) in Olive and Winarto *et al.* (2011) in *Anthurium*.

'Somaclonal Variation' refers to the variations observed among plantlets regenerated through callus culture (Larkin and Scowcraft, 1981). Isolation of somaclonal variation through callus phase is a potential tool for the geneticists and breeders, which permit development of new varieties with genetic variation (Maddock *et al.*, 1985, Seeta *et al.*, 2000 and Bairu *et al.*, 2011).

A repeatable protocol for isolation of somaclones was developed through callus phase of vegetative bud of turmeric variety Suguna (*Curcuma longa* L.). Somaclonal variation has a great potential for new

varieties with specific characters for plant breeding and commercialization.

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