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In-vitro Study of Somaclonal Variation in *Chlorophytum borivilianum*

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A B S T R A C T

Variations are genetically induced either *in vivo* or *in vitro* growth. The objective of the present investigation was to evaluate the effect of variations under *in vitro* growth in the *Chlorophytum borivilianum* an endangered medicinal herb. The chemically defined nutrient medium was act as stimulants to the explant and the growth response of regenerates were assessed. The MS media in combination with different growth regulators & pH (chemical factors) and some of the physical factors (external microenvironments i.e. variations in relative humidity, temp. and light/dark periods) was responsible for variations. The *in vitro* treatment, including different concentrations of the growth regulators were inducing agent. The long time in culture condition produced the greatest probability to initiate variations in clone lines under controlled environment. Three types of regenerates were recorded i.e. normal, vigorous, and dwarf. These variates were marked on the basis of visual score at the time of rooting. Medium compositions were act as elicitor and responsible for variations during the *in-vitro* multiplications. Due to impact of medium signal changes were recorded in the clones of the same propagules. The obtained mutant variety through *in vitro* selection was considered as somaclonals. These variations were recorded by visual scoring in their morphology. The variant shows morphological variations from their parental plant.

Introduction

Tissue culture is a source of genetic variability that gives rise to a phenomenon called somaclonal variation or culture-induced variation through genetic modification or micro-environmental conditions. Presence of sucrose and growth regulators in the medium induces heterotrophy or mixoheterotrophy in the cultures (Kozai *et al*., 1992). Different *in vitro* culture conditions contribute a culture induced phenotype in plantlets, which impedes their normal growth when transferred directly from culture to ambient greenhouse or field conditions and necessitates a period of acclimatization (Joshi *et al*., 2009). Recent studies have shown that the culture-induced phenotype can be modified towards autotrophy by reducing or completely eliminating sucrose in the medium (Nguyen *et al*., 2001). This concept of photoautotrophic micropropagation has recently been proposed as a means of reducing completely eliminating sucrose in the medium and

increasing CO_2 concentration (Joshi *et al*., 2009). Some of the environmental impact of non-living factors on the living organisms are the natural part of ecosystem that affect in a variety of ways on plant's growth which may be either beneficial or detrimental. When plants are exposed to a variety of different stress signals viz. drought, cold and salt each plant responds uniquely (Xiong *et al.,* 2002). Low temperature, drought and high salinity are common stress conditions that adversely affect plant growth and crop production. Plants evolved mechanisms to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope them. Tissue culture is a source of genetic variability that gives rise to variations through genetic modifications during the process of *in vitro* culture and this phenomenon is called somaclonal variation (Ehsanpour and Amini, 2008). The aim of the present study was to evaluate the degree of somaclonal variation by phenotypic analysis under different conditions and also to access the potential of somaclonal variation for development of novel cultivars in the endangered medicinal herb *C. borivilianum*. The plant is valued for its tuberous roots, which is being documented to possess immunomodulatory and aphrodisiac properties. The crop is fast disappearing from wild stands owing to its continuous exploitation and slow rate of perpetuation.

Materials and Methods

In the present investigation, the medicinal herb *Chlorophytum borivilianum* Sant. et. Fernand. (Liliaceae) was selected. The germplasms (healthy discs along with fingers and sprouted plantlets) were collected from two herbal nurseries of Bihar i.e. (i) IGIMS, Patna (ii) Muzaffarpur. IGIMS germplasm were selected on the basis of morpho-agronomical traits and used for *in vitro* studies. Shoot buds and floral stem were used as explants and surface sterilised separately. Washing in flowing tap water (20 min), followed by mixture of mild detergent (20-25 drops) each of Teapol, and savlon solutions, and rinsed thoroughly under tap water (3-5 min). The shoot buds were submerged in disinfectant solution of broad spectrum systemic fungicide and antibiotic [0.1% (w/v) Bavastin and 0.2% (w/v) Streptomycin] for 4 hrs. Under sterile conditions, decantation of solution and rinsing of shoot buds for several times with ddw was done. Now both the shoot buds and floral stems explants were dipped in (70%) ethanol (3-5 min), and then transferred to $HgCl_2$ solution. (0.05%) and (0.1%) were used for floral stem and shoot buds respectively for (2-3 min) in aseptic conditions. Finally rinsed thrice with sterile ddw. The pH of the medium was adjusted to 5.8 before sterilization. The surface sterilised explants (approx. 2 cm long) were inoculated aseptically on the MS media along with different concentrations growth regulators (Table.1). The cultures were maintained at 25 ± 2^0C, 16/8 hrs (light/dark) photoperiod and 65% humidity. Experiments were repeated three times to access the reproducibility of the results and subsequent evaluation of variates.

Results and Discussion

In vitro responses were observed for both the explants (shoot buds & floral stem) along with two combinations of media i.e. E_A and E_B media. Explant (floral stem) and E_B medium was superior than the shoot bud explant and E_A medium. The contamination in shoot bud cultures was more (35.60%) in both E_A and E_B media than floral stem (11.75%) and (11.60%) in E_A and E_B resp. Survival of *in vitro* cultures for shoot buds recorded was (62.05%) in E_A and (63.55%) in E_B. Survival in floral stem cultures was

better in E_B (85%) than in E_A (80%). Shoots are directly formed without callus formation and was high in E_B (61%) and only (58%) in E_A. Floral stem cultures formed somatic embryos (SEs) via, callus. (70%) and (82%) SEs were observed in E_A and E_B resp. From the observations it is quiet evident that E_B medium and floral stem explants gave better results than E_A medium and shoot bud explants[Fig.1]. (76%) cultures were recorded in the M_A and (82%) in M_B media. The number of shoots/explant obtained were (9.85) in M_A and (13.96) in M_B. Similarly, the number of leaves/tube scored were (11.05) and (14.05) in the M_A and M_B media resp. The observations on multiplication indicates that M_B medium is better than M_A with regard to culture response, no. of shoots, and leaves [Fig. 2]. (80%) rooted plantlets were recorded in R_A, while (91.64%) in the R_B media. No. of roots/pt. in R_B was more than R_A i.e. (15.26) and (11.20) resp[Fig. 4]. (60%) adventitious and (20%) tuberous roots were obtained in R_A, where as in R_B (52%) roots were adventitious, (28%) tuberous, and (11.64%) fibrous [[Fig.5]. The observations on rooting indicates that R_B medium is better than R_A with regard to root formation, type, and number. Rooted plantlets were treated with bioinoculant *Trichoderma harazianum.* The density of inoculums used was (2×10^6spores/ml), acclimatized in earthen pots, and (88.79%) plants were obtained. (85%) of plants were successfully established in the field.

Visual screening for variation was done for the field established plants. The variates were marked on the basis of their growth pattern and plant height, number, length, and width of leaves. Normal (74.5%), vigorous (8.9%), and dwarf (1.5%) plants were obtained [Fig. 5 &6]. Roots were also highly reduced in the dwarf group. The dwarf and vigorous plants were considered as somaclones. A wide variation in the investigated parameters was observed amongst the regenerates.

Growth response was analysed on the basis of certain parameters which includes growth pattern and plant height, number, length, and width of leaves. The morphological characters (variations) were noticed during multiplication stages (somatically dividing cells). These traits were remarkable in which few of the propagules were visualised as vigorous and someone as small. As widely known that variation obtained by tissue culture techniques provides a rapid and reliable approach for plant improvement.

Table.1 MS medium compositions

MS (Murashige and Skooge, 1962)								
Abbreviations	**Stock I**	**Stock II**	**Stock III**	**Stock IV**	**GRs (mg/l)**	**CM (%)**	**Sucrose (%)**	**Agar (%)**
E_A	MS Major salts	MS Minor salts	Iron EDTA Sol^n.	Vitamins	BAP (2)	10	3	0.8
E_B	MS Major salts	MS Minor salts	Iron EDTA Sol^n.	Vitamins	BAP (2) + 2,4-D(2)	10	3	0.8
M_A	MS Major salts	MS Minor salts	Iron EDTA Sol^n.	Vitamins	BAP (2) +2, 4- D (2)	10	3	0.8
M_B	MS Major salts	MS Minor salts	Iron EDTA Sol^n.	Vitamins	BAP (4) +2, 4-D (2)	10	3	0.8
R_A	½ MS Major salts	MS Minor salts	Iron EDTA Sol^n.	Vitamins	BAP (2) + IAA (1)	5	3	-----
R_B	½ MS Major salts	MS Minor salts	Iron EDTA Sol^n.	Vitamins	BAP(2) +IBA (1)	5	3	------

Fig.1

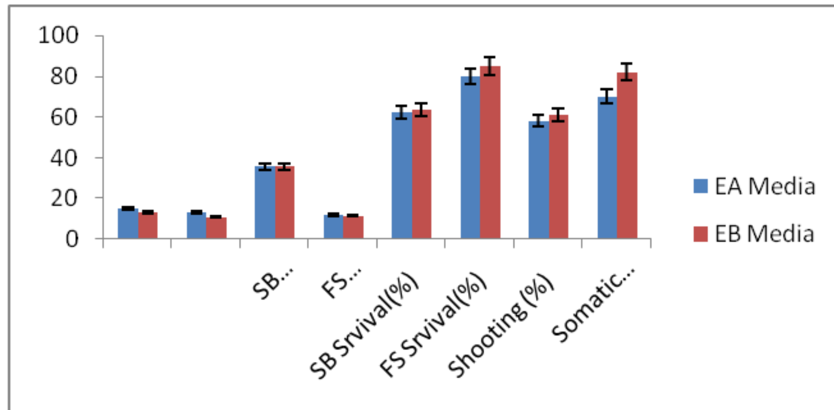

Fig.2

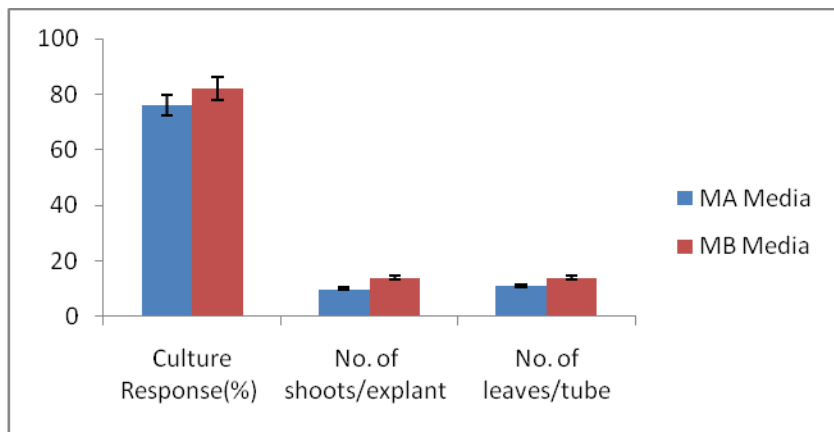

Fig.3

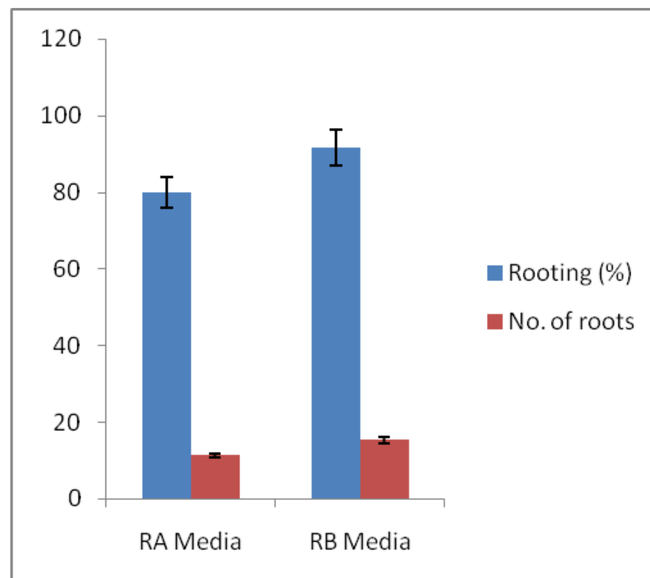

Fig.4

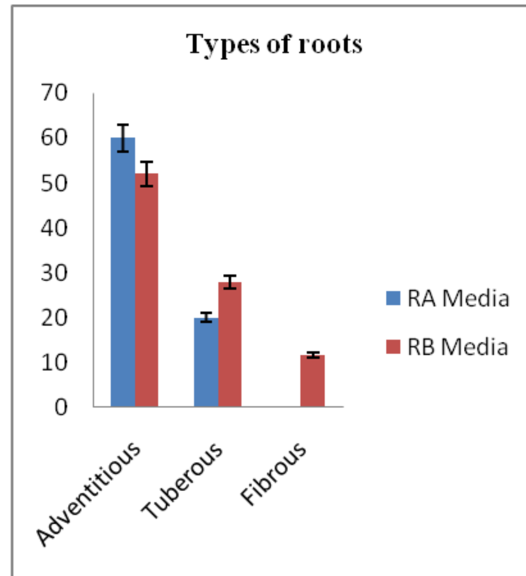

Fig.5

Fig.6

Phenotypic evaluation based on morphological traits of elite plants for every salt treatment is one of the most popular methods for the detection of somaclonal variations (Anwar *et al.,* 2010). These changes in morphology is manifested as cytological abnormalities, frequent qualitative and quantitative phenotypic mutation, sequence change, and gene activation and silencing through the modulation of gene function. Activation of quiescent transposable elements and retrotransposons indicate that epigenetic changes occur through the culture process (Kaeppler *et al.,* l.c.). Lang *et al.,* (2002) remarked that the variations may be due to chromosomal aberrations, DNA amplification, and the presence of transposable elements. The chromosomal and gene mutations discovered in somaclonal variants may already be present in the plants or they may be regenerated during the *in vitro* culture. Somatically stable variation includes phenotypes such as habituation of cultures and physiologically induced variations may be observed among primary regenerates and is often not transmitted to the subsequent generations (Patterson *et al.*, 1993; Cubas *et al.*, 1999). The identification and development of salt tolerant genotypes is of utmost importance for adaptation to the changing environmental conditions (Hasegawa *et al*., 2000; Koiwa *et al.,* 2006; Munns and Tester, 2008). Application of osmotic stress under *in vitro* condition is effective and ideal for the selection of tolerant variety as it can be carried out under artificial controlled conditions with limited space and time (Mitler, 2006). A wide variation in the investigated parameters were recorded amongst the regenerates, tested thrice. The results revealed that the selected parameters varied significantly with respect to the normal plant. The occurrence of somaclonal variation is a matter of great concern for any micropropagation system and studies on it are important tool which will enable breeders for genetic improvements. In nature, the genetic diversity and variability within a population are generated via recombination events. Factors such as natural selection, mutation, migration & population size along with environmental conditions influence genetic variability in different ways. Plant cells cultured *in vitro* showed a genetic instability that was also a characteristic of regenerated cells. In vitro, the conditions of culture can be mutagenic and the regenerated plant derived from it sometimes can show phenotypic and genotypic variations.

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