BIOGLOBIA Vol. 7 (2): 69 –73, 2020 **ISSN: 2349-5626**

Screening and Selection of Better Genotype Through *In-vitro* Culture in *Asparagus racemosus* Willd (Liliaceae)

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Received: 30.07.2020 **Revised: 25.08.2020** **Accepted: 23.09.2020**

ABSTRACT

Asparagus racemosus is a medicinal plant. The dry tubers are essential constituent of several herbal product used in various kind of diseases. In this study the principle was to screening of regenerates as possible variants from parental stock. The plant is medicinally important so it is desirable to screen variants useful for agronomic traits. The propagation of plants through in vitro culture imparts them ability to produce variants different from parental stock. Nodal segment selected as explant were inoculated in seven different MS media composition with GR IAA, BAP, 2, 4-D, NAA & kin at the concentration of (2.0- 2.60 mg/l). 79.12% nodal explant responded with (KIN 4.0, 2,4-D 3.0 mg/l) after 45 days of inoculation. 75.5%calli formed shoots in media with composition with BA (2.5) + IAA (2.0) +NAA (2.0) after 30 days .85.2% rooted plantlets were acclimatised and 76.34% were successfully established in the field. In this study the principle was to analyse regenerates in vitro for possible variants from parental stock. Three month old plant were analysed for somaclonal variation. 98.2 %plants were similar to parental stock.1-2% plants showed variation in the form of size and numbers of tubers. The variant tubers attained length up to 15.1± 0.9 cm with width 0.61±0.02 cm. The no of tubers/stock also varied from 25±1.0 to 31.04±1.5. The increased no of tubers and their length would be beneficial for agronomic traits. Most of the plants (98.2%) showed resemblance towards parental stock. Rest of the plant showed variation in the form of dense and scarce cladode, larger internode size varied colour of tubers , Size (L&W) of tubers. The approaches which were used in the study would be reliable and easy to detect somaclonal variation at morphological parameters. Which is needed to be verified at other parameters.

The present study shows examples of some useful variants marked as somaclones through *in-vitro* condition varied from parental stock.

Keywords: *Somaclonal variants, Screening of regenerates, Nodal segment, Morphological parameter, GRS.*

INTRODUCTION

Asparagus racemosus Willd (Liliaceae) is an important medicinal monocot plant of tropical and subtropical India. The plant is small perennial woody climber. The underground rhizome contains tubers of pharmaceutical properties used in various diseases related with female reproductive system, menopause irregularity and immune system modulator, cholinesterase activities, antioxidant properties. Ojha *et al.*, (2010).It is known for its phytoesterogenic ,anticancerous, antiulcerogenic, antioxidant, anti-ADH, activities (Bopana and Saxena, 2007).The tubers of the plant contains active constituents like steroidal Saponins, Shatavarin 1-4, Oligospirostanoside, Asparagine, Racemosol, Carbohydrate, Flavenoids, Isoflavones, Asparagenine polysaccharides ,essential fatty acids and minerals which provides them therapeutical potential used in memory enhancer, aphrodisiac, antiaging, diarrhoea, nervarine tonic, miscarriage *etc.* (Wiboonpun *et al.*, 2004) and Jadav and Butnaik., (2006).The propagation of plants through in-vitro culture imparts them ability to produce

variants different from parental stock and to overcome low germinating rate through seeds. Random genetic variability occurring in plant tissue culturemay trigger better agronomic traits that may not be achieved by conventional breeding methods. Variation in clones during in-vitro culture is known as somaclonal variation. which has been employed in agronomy. The improvement of crops through creation of noval variants has been documented by several workers. Mehta and Angra (2000), Paradien, (2001) and Wiboonpun *et al*., (2004) isolated a new antioxidant compound named racemofuran along with two other compounds namely asparagamine A and racemosol from root and rhizome which exhibited antioxidant activity against DPPH. It is used in about 66 Ayurvedic formulations. (Nandkarni,1954 and Srikanta, 1997 and Sharma and Dash, 2003). It is desirable to grow plants through *in-vitro* culture which have low chances of germinating and growing through seed like *Asparagus racemosus* and to detect variation which is reflected superficially (Morphological parameters). It is one of the most vivid parameters which is needed to be get supported at other parameters.

MATERIALS AND METHODS

The seeds are obtained from Indira Gandhi Institute Of Medical science (IGIMS) Herbal Garden Patna. Germplasms were multiplied under controlled environmental condition in the poly house and maintained in the research garden Department of Botany Patna University. After 30 days seedlings came out. The nodal segment were selected as explant from three month old plant.

Surface sterilization:

The explant was sterilized by washing in tap water (20min) followed by mixture of mild detergent (20-25 drops) of teepol and savlon solution rinsed thoroughly under tap water(3-5mins) dipped in 70% ethanol(3-5min) and then transferred to 0.05% Hgcl2 soln (1m). And 2-3mins rinsed thrice with sterile ddw under Laminar air flow cabinet.

Culture initiation:

Surface sterilised explants were inoculated aseptically on to MS media. Multiplication and rooting were achieved with different concentrations of GRS. The culture were regularly observed and data were recorded up to seven weeks.

Up to three month field established plants were monitored for variations.

Screening of regenerates for Soma clonal variations:

The screening of regenerates for morphological traits started from multiplication stage and continued upto field establishment. Due weightage was given to the following parameters:

1. Habit of the regenerates (Normal, Plants with long internodes, and Plants with short internodes)
2. Cladode arrangement on the node (dense/scarce)
3. Cladode color (Dark/ Light) green
4. Internode length
5. Colour of tubers
6. Number of tubers /crown

Table 1

Culture Response of Explant Nodal Segment After(45 days) of Inoculation

Diff stages of Culture	Media	GRs	Survival
Culture initiation	MS	Kin(4.0)+2-4,D(3.0)	79.12%
Multiplication	MS	IAA(1.25)+BAP(2.5)	75.5%
Rooting	MS1/2	BA(2.5)+IAA(2.0)+NAA(2.0)	75.5%
Acclimatization	85.2%		
Field survival	76.34%		

Three month old field established plant is analysed at morphological levels to detect variants from parental stock.

Table 2

Somaclonal variations observed in 3months old plant

Habit of plant	No of Tubers /Stock	Length of tubers(cm)	Width of tubers(cm)	Colour of the tuber	Size of the internode(cm)
Doner	25±1.2	2.06±0.7	0.26±0.3	Brownish	1.5±0.3
Scarcy	30.4±1.5	14.5±1.1	0.61±0.04	Whitish	1.82±0.1
Vigourous	31.0±11.5	15.1±0.9	0. 69±0.02	Whitish	1.75±0.3

Fig. 1. Showing general habit of the plant

Fig. 2. Tuber of the Doner plant,

Fig. 3. Brown Regenerate,

Fig. 4. White regenerate

RESULTS AND DISCUSSION

Shoot tips were inoculated in seven different MS media composition with IAA, BAP,2 4-D,NAA and KIN and multiplied in eight diff composition of IAA, BAP,2 4-D,NAA and IBA at the concn of (0.5- 4.0 mg/l). 79.12% nodal explant exposed in IN7.After 45 days of inoculation.75.5% formed shoots in m4 media after 30 days. Healthy shoots (2-6cm) were then transferred to liquid rooting media (R1-RR3) with diff concentration of GR namely IBA, NAA at the concentration of (2.0-3.0 mg/l). Maximum no of roots formed in R3 media after

40 days.75.5% with optimum root length (2.05cm) were scored.85.2%rooted plantlets were acclimatised and 76.34% were successfully established in the field. Maximum field established plants 98.2% showed resemblance with parental stock but 1.8% plants depicted variation from the parental stock which has been recorded at the morphological parameters like cladode arrangement varied from dense to scarce, the variant plant have long internode with long and thin cladode. color of the tubers varied from creamy to whitish. No of tubers found to be more in no *i.e* 30 .4±1.5 to31.01±1.5 per stock. whereas in parental stock it is approximately 25±1.2.The length of the tuber get increased from 2.06±0.7 to15.1±0.9cm whereas the width get decreased from 0.26 ±0.3cm to 0.69±0.04cm.The internode size appeared to be larger i.e 1.82 ±0.1cm in the variant. whereas in parental stock it is recorded as1.5±0.3cm. The variant with useful agronomic traits has to be stabilized for large scale commercial application. Some plants depicted creeping habit. The plant *Asparagus. racemosus* is widely used for it's pharmaceutical properties present in tubers and for ornamental purposes. In this study *A. racemosus* is selected for detection of variants as Somaclone for commercial value. Somaclonal variation may reflect pre exising cellular genetic differences or tissue culture induced variability. *In-vitro* regenerates have potential agriculture and horticulture significance. The size of the tubers showed variation from parental stock with useful agronomic trait. Permanent variant referred as somaclonal variants are heritable and often represented an expression of pre-existing variation in the source plant (Larkin and Scowcroft, 1981). In this report the somaclonal variant is detected at morphological parameters. In date palm the production of bastard offshoot, excessive vegetative growth, leaf whitening and variegation are common morphological traits used in detecting somaclonal variant (Zaid and Al Kababi, 2003). First observation and report of somaclonal variation is by Brun, 1959, however permanent variations referred to somaclonal variation are heritable andreversible (Keppler *et al*., (2000). It is useful in crop improvement through criterion of novel variants are also well documented (Mehta and Angara, 2000; and Predieri, 2001). Induced somaclones may be useful for genetic manipulation of crop with polygenic traits (Brar and Jain, 1998 and Jain, 2001). Phenotypic variants included aberrant flowers and cladodes, larger flower size and glaucose foliage in *Asparagus racemosus* plant regenerated by organogenesis from long term callus culture (Pantaroli and Camadro., 2005). It has been observed that the frequency of expression of dominance is higher in somaclonal variation than in other forms of mutation.(Wiboonpun *et al*., 2004; Sharma *et al*., and Yang *et al*., 2010). Size is getting increased in present case may be due to result of modification or mutation in the expression of gene involved in tuber development. The approach which were used in this study would be reliable and easy to detect somaclonal variation at first glance. The variations are considered as a new source for enlarging and enriching the gene pool of important varieties (Bairu *et al*., 2011). Somaclonal variation may reflect pre exising cellular genetic differences or tissue culture induced variability. *In-vitro* regenerates have potential agriculture and horticulture significant. Lomror *et al*., has developed somatic embryos in the *in vitro* culture of zygotic embryos and hypocotyl seedlings of *Asparagus racemosus*. The study has provided first hand information to select variation at morphological levels which need to be ascertained at other parameters. The approaches used in this study is helpful for assessing somaclonal variation at morphological level. The probable useful variability need to be enhanced and would be exploited for commercial purposes.

CONCLUSION

In–vitro condition provide atmosphere to surface the pre-existing variation which is not vivid through general breeding methods. These variation could be detected morphologicaly and need to be ascertained at other parameters**.** The approach selected in this study seems to be proved as reliable for assessment of somaclonal variation in *Asparagus racemosus* could be exploited for commercial purposes.

ACKNOWLEDGEMENTS

I am thankful to Head of the Botany Department for providing Tissue Culture lab facilities. I am greatly obliged to my mentor Dr. Chander Prabha (Associate Professor of Botany, Patna Science College, Patna University, Patna, Bihar) for her benevolent presence and guide.

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