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DEVELOPMENT OF *IN VITRO* REGENERATION PROTOCOL IN GROUNDNUT CULTIVAR 'DHARANI' USING COTYLEDONARY NODE EXPLANTS

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Abstract

The groundnut cultivar 'Dharani' grown in the state of Andhra Pradesh, India is one of the locally available varieties. *In vitro* tissue culture protocols are very scanty for this cultivar and it is the time to develop regeneration system through advance biotechnological methods to improve the crop quality. In the present attempt,cotyledonary nodal explants were used to develop the direct plant regeneration protocol. Different types of media along with plant hormones were employed in different concentrations and combinations. Multiple shoot bud initiation and percent explants responded were observed at frequent intervals. The maximum explants were responded on Murashige and Skoog (MS, 1962) medium supplemented with 2.0mg/l BAP (6-Benzylaminopurine) along with 0.5mg/l of NAA (1-Naphthaleneacetic acid) with 4 to 5 multiple shoot production. In addition, rooting was also achieved for *in vitro* regenerated shoots in MS with different concentrations of NAA. The well rooted plants were acclimatized to greenhouse conditions.

Keywords: Dharani, Regeneration, MS Medium, BAP, NAA, Rooting.

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INTRODUCTION

Groundnut or peanut is a major oilseed crop and is cultivated in different regions of the world. Apart from oil food source, it is also used as fodder for animals. More than 25.2 million ha cultivated globally with a total production of 41.2 million tons with an average yield of 1.67 tons per ha. Peanut is the second largest crop in India and yielding an average of 8 million tons from 6 million ha which is expected to reach up to 14.8 million tons by the year 2020 to meet the rising demand [1]. Recently the share of groundnut was lost severely due to both biotic and abiotic factors which are the main threat to yield. So it is necessary to improve groundnut growth and yield through breeding or genetic engineering methods. Before that, it is necessary to establish *in vitro* tissue culture protocols for different groundnut cultivars. Dharani cultivar is popular especially in Andhra pradesh because of its reasonable yield. Although this cultivar has a good impact on agriculture, further yield improvement of this variety has been neglected. In addition, most of the other cultivated varieties of groundnuts are also sensitive to biotic and abiotic stresses such as salinity, drought, extreme temperature, bacterial, fungal and viral diseases [1, 2]. Application of genetic transformation technique may also helpful in improvement of biotic or abiotic stress tolerance in this crop. As mentioned above establishment of *in vitro* regeneration system is prerequisite before genetic improvement [3]. Different research groups developed the methods for *in vitro* regeneration protocols for various groundnut cultivars [4, 5, 6, 7, 8]. But information is cultivar specific and there was no specific *in vitro* regeneration protocol in Dharani variety till date. In some cases the response of *in vitro* plant regeneration protocol is genotype-dependent [9]. We previously worked with groundnut to check antibacterial and antioxidant properties using leaf extracts [10]. So in the present study, an attempt was made to develop the efficient *in vitro* regeneration system in this cultivar.

MATERIALS AND METHODS

PLANT MATERIAL: Healthy and mature seeds of Dharani cultivar of peanut were obtained from the Agricultural Research Center, Utukur, Kadapa, Andhra Pradesh, India for the present study. After collection, stored at 10^0C for further usage.

PREPARATION OF COTYLEDONARY NODAL EXPLANTS: Mature groundnut seeds were surface sterilized in 70% ethanol for 1min and then in 0.1% mercuric chloride solution for 6-8min, followed by six times rinsing using distilled water. Later all the sterilized seeds were germinated on water agar medium which contains 0.8% agar. Five to seven day old seedlings

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were selected for preparation of cotyledonary node explants and were used for *in vitro* regeneration.

DIRECT REGENERATION : The prepared cotyledonary nodal explants were used for the induction of multiple shoots. Initially, the explants were transferred to different media prepared with various concentrations and combinations of plant growth hormones. We narrow down the protocols and finalized the multiple shoot induction medium for this particular cultivar. Multiple shoot induction medium consists MS salts (1962) [11] along with different concentrations and combinations of growth regulators such as BAP and NAA (Table-1). Generally, 3% sucrose and 0.8% agar were used for the present study and pH was adjusted to 5.8 before autoclaving the media. Multiple shoots generated were separated and kept for subculture medium which consist MS salts with BAP alone and along with low concentration of NAA. After 3-4 weeks of the incubation period for shoot elongation, the healthy elongated shoots were transferred to root initiation medium supplemented with MS salts along with NAA alone or in combination with low concentrations of BAP for another 3-4 weeks. The well rooted plants were washed and transferred to pots containing autoclaved soil and later transferred to glasshouse.

All the cultures were visually observed frequently in culture room and the statistical work has been done using excel program in the personal computer.

RESULTS AND DISCUSSION

In the present investigation, the cotyledonary nodal explants were obtained from five to seven day old *in vitro* germinated seeds on water agar medium for further experiments (Fig.1).

All the inoculated cotyledonary nodal explants showed multiple shoot initiation within a week on MS medium in a combination of BAP and NAA.

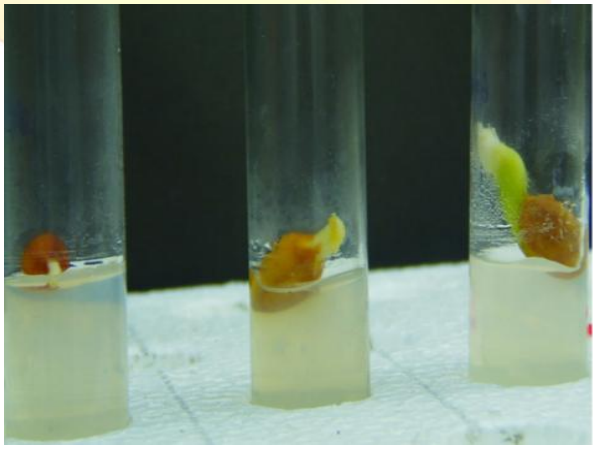

Fig.1: Seed germination on water agar medium showing seven day old seedlings

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MS medium without any hormones does not give multiple shoots and at the same time, a high concentration of BAP also resulted only one or two shoots in the culture medium. Similarly, Sujatha et al. [12] also proved that the hormone-free medium exhibits no morphogenesis with *Pongamia pinnata*. The contribution of cytokinins on shoot bud formation has been extensively studied in groundnut as well other species [13, 14, 15]. The maximum explants responded in MS medium along with 2.0 mg/l BAP and 0.5mg/l NAA combination and all were a healthy green in colour (Table-1 and Fig.2). The induction of 4 to 5 multiple shoots was achieved in the same medium only (Fig. 2).

Table 1: Multiple shoot induction using cotyledonary nodal explants

MS medium with plant growth regulator (mg/l)		Number of explants responded (in percentage)	Number of multiple shoots/explant (SE)
BAP	NAA		
0	0	53.33	1.0±0.00
0.1	0.5	55.00	1.0±0.00
0.5	0.5	58.33	1.3±0.06
1.0	0.5	66.66	1.8±0.11
1.5	0.5	68.33	3.0±0.00
2.0	**0.5**	**71.66**	**4.6±0.11**
3.0	0.5	65.00	2.0±0.00
5.0	0.5	58.33	1.6±0.06

Above data indicates mean of 20 replicates and experiment conducted thrice

The multiple shoots were excised and individual shoots transferred for further shoot elongation. After the successful subcultures, the green and healthy elongated shoots were transferred to rooting media. Apart from all combinations and concentrations tested MS with NAA alone showed the highest frequency of rooting (Fig. 3).

Fig.2: Multiple shoot regeneration from cotyledonary node explants of groundnut cultivar Dharani (MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA)

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Initially, the root buds appeared as a cluster after 2 weeks of the incubation period. Later they were fully developed into mature roots within 4 weeks. The rooting of *in vitro* regenerated shoots in media without plant hormones showed negligible results (data not shown). Rooted plantlets transferred to pots filled with autoclaved soilrite and hardened in the glasshouse. The plants growing healthy and they appeared phenotypically normal in the glasshouse.

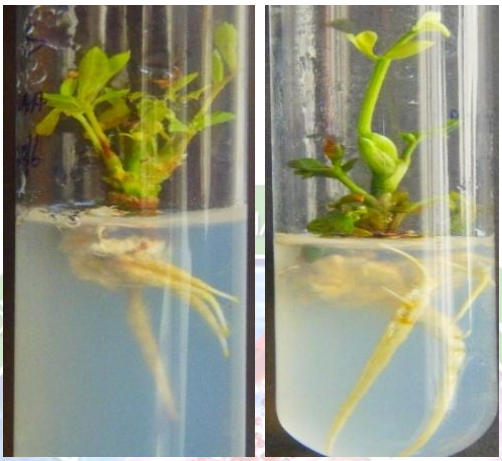

Fig. 3: Root initiation from *in vitro* regenerated shoots

Previously extensive work has been done on various cultivars of groundnut regeneration and direct organogenesis using different type of explants [5, 8, 16, 17, 18, 19]. But here in the present study, a simple standardized high frequency regeneration protocol has been developed using cotyledonary node explants in Dharani cultivar. In addition, MS with NAA suited for the high frequency of rooting and similar results also reported on groundnut with slight differences [Fig.3] [20].The rooted plants transferred to glasshouse and grown normally without any morphological differences. Generally, the response i.e. induction of multiples shoot buds is genotype or cultivar-specific and also depend on media provided [9]. So the present data has also successfully tagged to the growing body of knowledge, especially for groundnut cultivation.

CONCLUSIONS

In conclusion,present study stated that MS with BAP (2.0 mg/l) in combination with NAA (0.5 mg/l) triggers the multiple shoots in Dharani cultivar. This efficient regeneration protocol may be useful for the genetic engineering experiments to produce transgenic lines to improve the tolerance for both biotic and abiotic stresses and ultimately for yield.

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