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Evaluation of Indian Peanut on the Induction of Caulogenic Buds *in Vitro*

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Abstract The present work has been undertaken to study the *in vitro* response in widely used cultivars WesternHB-55, TAG 24 and SB11 of peanut having desired agronomic characteristics. The mature embryo derived leaflet explants of all three peanut genotypes were found morphogenic *in vitro*. When comparing the effects of TDZ and BA with respect to caulogenic response, it can be inferred that TDZ is a more potent inducer of caulogenesis *in vitro* than BA. All genotypes responded equally to different concentrations of BA. WesternHB-55 responded better than others which results in high proliferation rate *in vitro*. The present study emphasizes on the usage of WesternHB-55 germplasm through adventitious organogenesis pathway in transformation studies (biolistic gun approach), for the production of proteins, vaccines enzymes etc.

Keywords Peanut, Westernhb-55, TDZ, Adventitious, Organogenesis, TAG 24, SB 11

1. Introduction

Peanut (*Arachis hypogaea* L.) is the major oilseed crop of India. India is the second largest peanut producer after China. Many of the important agronomic traits high yield and quality, earliness, resistance to major pests, diseases, high protein and oil content have yet to be improved through gene transfer technology[1]. To exploit the existing germplasm for improvement, various genotypes need to be studied for their desired agronomic characteristics as well as their *in vitro* response. *In vitro* regeneration, a key requisite in the development of transgenic peanut plants is highly genotype dependent. Differences in the *in vitro* response among the non Indian genotypes belong to Spanish, Runner, Virginia, and Valencia types had been extensively studied and reported [2]. Though in India, more than a hundred varieties of peanut are released for cultivation based on its agronomic importance (SB11, JL 24, WesternHB-55, TAG 24, ICGS 1, GG 20, K134 etc), literature pertaining to *in vitro* studies is very much limited to SB11 and JL 24. Cultivars like TAG 24 (semi dwarf, early maturing and high yielding), WesternHB-55 (early maturing and high oil content) belong to Spanish type with desired agronomic characteristics are widely used in the central region of India, but their germplasm was not exploited for peanut improvement. Chengalrayan et al. studied the genotype-specific response at each stage of somatic embryogenesis in 16 Indian genotypes including SB- 11 and TAG 24[3]. However, there are no reports available on the genotype-specific response in the direct adventitious organogenesis pathway. This pathway is useful in gene transfer techniques and well suited for many transient expressions involved in the production of proteins, enzymes, vaccines etc[4]. Differences in response observed *in vitro* by peanut genotypes is possibly due to the complex interaction of genes controlling morphogenesis in the genotype with the culture protocol[5]. Irrespective of the pathway, the major difference lies in the initial induction phase.

Hence, the present study was carried out to evaluate the agronomically important peanut cultivars belong to Spanish groups like TAG 24, WesternHB-55 including SB 11 as control, for the direct induction of caulogenic buds in mature zygotic embryo derived leaflets. Also, the interaction of plant growth regulators during induction phase was studied.

2. Materials and Methods

Pods of mature seeds of three cultivars of peanut were collected in the month of December from Agricultural College, Pune, India. WesternHB-55 is early maturing high oil yielding cultivar. TAG 24 is a semi dwarf early maturing and high yielding groundnut variety. Its pedigree is TGS 2 x TGE 1 and is of the Spanish variety which has a high harvest index and is tolerant to bud necrosis disease[6]. The yield is 2000 kg/ha. The cultivar SB11's pedigree is Ah 4213 x Ah 4394, belong to Spanish variety. The dates of release are 1991 and 1965 respectively[6].

To initiate the cultures, the embryoaxes from the dehusked seeds of TAG 24, SB 11 and WesternHB-55 were excised. Then it was rinsed with tap water twice after the addition of

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two drops of 1% liquid detergent. Mercuric chloride treatment (0.1%) treatment was given for 2 min. followed by rinsing with sterile distilled water for 4-5 times and kept in little sterile water for 12-16 hrs (over night incubation) and cultured on to MS basal medium for germination. The explants were incubated in dark for 3 days and transferred to tubes. Data on shoot length root length and number of leaves were collected after 30 days of culture. Thirty explants per treatment were taken and the experiment was repeated thrice.

To induce direct adventitious organogenesis, the explants were sterilized as mentioned but after overnight incubation in sterile distilled water for the opening up of the MZEDL, the leaflets were carefully excised without any damage and were cultured on to MS medium supplemented with different concentrations of BA and TDZ (4.4 µM, 13.2 µM, and 22 µM) with MS basal medium as a control. Fifty explants per treatment were tested and the experiment was repeated thrice.

Data on caulogenic bud induction was collected after 30 days of culture. All cultures were incubated to 25 ± 2°C light conditions in irradiance of 32 µmol $m^{-2}s^{-1,}$ 16 h photoperiod. For optimization parameters, Completely Randomized Designs were used. The data was subjected to analysis of variance (ANOVA) and treatment means were compared using F test[7]. To differentiate morphogenic cells from non-morphogenic cells, a double-staining procedure described by Gupta and Holmstrom was followed[8].

3. Results and Discussion

Before studying the morphogenic responses, it is imperative to determine viability of the seeds by germinating *in vitro*. Instead of seeds, embryo axes were the explants used in order to minimize the occurrence of contamination i.e. to establish an aseptic culture. Radical emergence was considered as sign of germination. The genotype TAG 24 showed the highest germination frequency with approximately 90% (90.0 ± 9.43) of the explants inoculated had germinated while SB 11 and WesternHB-55 showed equal frequency of about 73% (73.3 ± 14.1 & 73.3 ± 1.00). Germination parameters like shoot length, root length and number of leaves showed no significant difference in response among the genotypes (Table 1).

Table 1. Germination parameters of peanut genotypes

Genotype	Parameters (Mean± s.d.)		
	Shoot Length	Root Length	Number of Leaves
SB 11	2.8± 1.82	2.12± 1.23	7.22± 3.46
WesternHB-55	2.5± 1.52	1.69± 1.25	9.60± 14.42
TAG 24	1.5± 2.37	2.98± 1.69	8.78± 4.11
F-value	**NS**	**NS**	**NS**

One of the remarkable feats of earlier physiological analysis on *in vitro* organogenesis was the identification of the predominant role of auxin and cytokinin as chemical determinants in plant development. It has been reported that these cytokinins promoted shoot regeneration from explants of most of the grain legumes[9]. The effect of BA on shoot bud induction was attributed to the ability of plant tissues to metabolize the natural hormone more readily than other, synthetic, growth regulators or to induce endogenous production of zeatin. It is an efficient cytokinin and is commonly used in plant tissue culture.

In the present study, supplementation of BA in MS media, induced caulogenic buds in MZEDL explants in all the peanut genotypes under study. Definite and significant pattern of response was obtained in all the genotypes. Maximum number of buds was produced in the MS media fortified with 13.2 µM BA, in all three genotypes. Further increase in concentration of BA (22 µM) resulted in low response. Hence, these genotypes responded equally in terms of inducing caulogenic buds with reference to BA (Fig. 1). Unpredictably, one or two caulogenic bud like induction was noted in explants cultured in MS media without PGR (control). Callus was not observed at this stage of culture and all caulogenic buds seemed to develop *de novo* i.e. direct adventitious organogenesis. Callusing was observed in control. It is therefore evident in this study that BA is capable of inducing caulogenic buds in these groundnut varieties and the genotypes responded similarly. Banerjee et al. (2007) evaluated the shooting potential among five different peanut cultivars and obtained a variable response among the cultivars and reported that it might be due to change in endogenous PGR level[10]. Our result suggests that protocol followed for caulogenic bud induction in SB 11 can be applied to TAG 24 and WesternHB-55 and similar results can be expected. Hence, the cultivars like TAG 24 and Western-55 can be applied as a model system for gene transfer techniques.

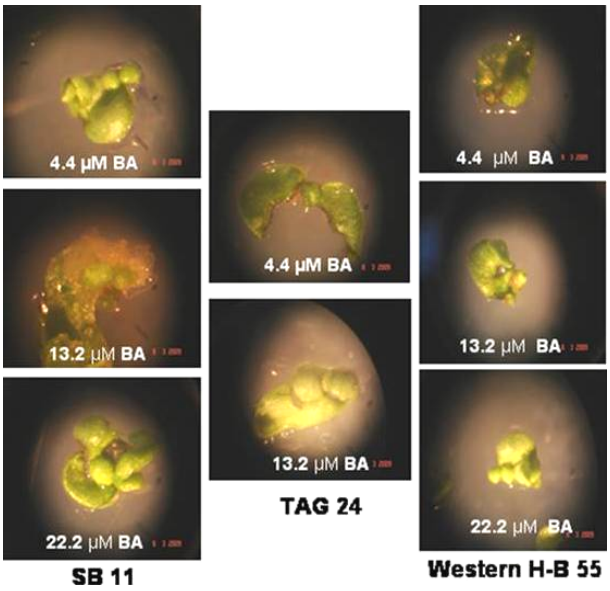

Figure 1. Caulogenic bud induction in MZEDL explants of peanut genotypes with different concentrations of BA

Table 2. Caulogenic bud induction frequency in different concentrations of BA among genotypes

Concentration (µM)	Frequency of bud induction in Genotypes (%)		
	SB11 (Mean± sd)	TAG 24 (Mean± sd)	WesternHB-55 (Mean± sd)
0	0.04± 0.05	4±5.6	8± 5.6
4.4	44± 11.3	16± 0	30± 25.4
13.2	42± 14.1	44± 5.6	46± 25.4
22.2	36± 22.6	4±5.6	18± 14.1
F-value	**	**	**

**, P≤0.01

TDZ is a potent cytokinin that promotes shoot formation and plant regeneration via organogenesis and somatic embryogenesis in cultivated peanut within a short duration while in culture[2,11,12]. Visser et al. (1992) first described the effective substitution by TDZ of both auxin and cytokinin requirement of the geranium tissue culture system[13]. Later studies confirmed the effective use of TDZ as the sole growth-regulating compound for the induction of regeneration in many species like *Phaseolus vulgaris[*14], Peanut[15,16], *Cicer*[17] etc.

In the present work, TDZ was found to be effective in inducing caulogenic buds in MZEDL explants of peanut (Table 3). WesternHB-55 produced more caulogenic buds (60 ± 6.3) even at low concentrations of TDZ (4.4 µM) and with the increase in concentration the response decreased. TAG 24 showed high caulogenic response in 13.2 µM and SB 11 at 22.2 µM (Fig. 2), respectively.

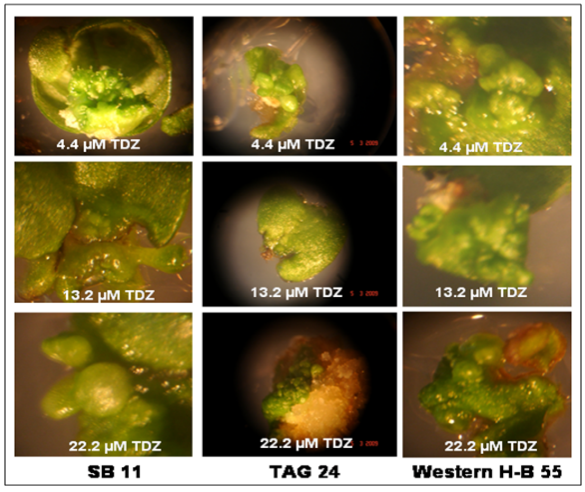

Figure 2. Effect of different concentrations of TDZ in caulogenic bud induction in MZEDL explants of peanut genotypes

Table 3. Caulogenic bud induction frequency in different concentrations of TDZ among Genotypes

Concentration (µM)	Frequency of bud induction in Genotypes (%)		
	SB11 (Mean± sd)	TAG 24 (Mean± sd)	WesternHB-55 (Mean± sd)
0	0	10± 2.8	20± 5.6
4.4	45± 35.3	36± 16.9	60± 6.3
13.2	40± 5.6	46± 2.8	38± 2.8
22.2	54± 2.8	42± 25.4	26± 19.7
F-value	**	**	**

**, P≤0.01

Unpredictably, caulogenic bud like induction was noted in the explants cultured in MS media without PGR (control) of TAG 24 as well as that of WesternHB-55. Callus was observed at this stage of culture in some explants but all caulogenic buds seemed to develop *de novo* i.e. direct organogenesis. Control explants showed callus significantly. Another report by Matand and Prakash(2007) was that the use of TDZ (0.5 mg/l) for 10 days appeared to be sufficient to promote shoot formation in explants across peanut botanical groups[11].

In this study, the concentrations of TDZ used here were the equivalent concentrations to that of BA so that a potential comparable data can be obtained. TDZ induced organogenesis at much lower concentrations via a reduced dominance of the apical meristem, resulting in formation of adventitious and/or axillary buds and ensure the proper development of plantlets without any impaired growth[16]. It is interesting to note that WesternHB-55 responded well in the lower concentration of TDZ in comparison to SB 11 and TAG 24, thus making it very effective in producing more caulogenic buds as well as their proper growth and development of plantlets.

Microscopic observation of double-stained normal and morphogenic mass (caulogenic buds) showed that the cells from morphogenic masses were very large compared to normal cells (Fig. 3). They had large nuclei and stained bright red by acetocarmine compared to control. The cytoplasm and vacuole of some cells are also stained by acetocarmine, confirming the caulogenic status of the cells. The exclusion of Evan's blue stain by the normal and morphogenic cells confirms the viability of these cells.

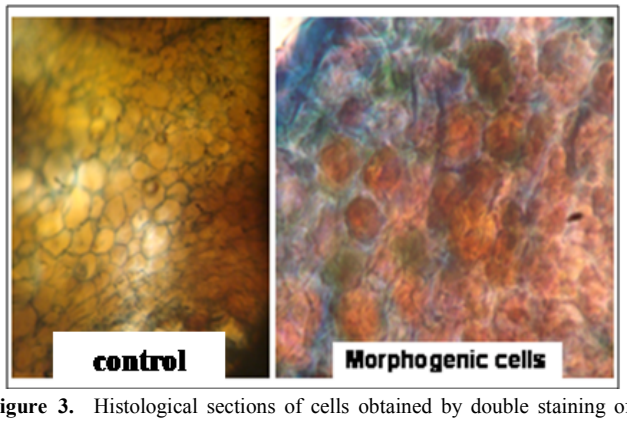

Figure 3. Histological sections of cells obtained by double staining of cells in control and morphogenic mass obtained in TDZ

4. Conclusions

It can be stated from this study that Spanish varieties TAG 24 and WesternHB-55 have *in vitro* morphogenetic potential similar to SB 11. Inoculating the MZEDL of these varieties in MS media fortified with the growth regulators BA or TDZ induced caulogenesis in all genotypes, which appeared to be direct *de novo* organogenesis in BA. Indirect caulogenesis in TDZ was observed to some extent however direct caulogenesis was mostly observed. The morphogenic nature of

the cells was confirmed with histological staining methods.

When comparing the effects of TDZ and BA with respect to caulogenic response, it can be inferred from these values that TDZ is a more potent inducer of caulogenesis *in vitro* than BA by producing more buds. All three genotypes responded equally to different concentrations of BA. In the MS media supplemented with varied concentrations of TDZ, the variety WesternHB-55 responded better than SB 11 and TAG 24 in terms of number of buds. Its maximum response at lower concentrations of TDZ (4.4 µM) ensures the proper growth avoids the impairment of growth due to high exposure. Among the Spanish varieties only SB 11 has been exploited for transformation studies.

The present study emphasizes on the usage of other cultivar like WesternHB-55 whose *in vitro* response similar to SB 11 in tested BA concentration and better than SB 11 in TDZ supplemented media. The explants of WesternHB-55 produced caulogenic buds through direct organogenesis pathway compared to other germplasm. These explants can be targeted through biolistic gun delivery for desired protein or vaccines expression in their caulogenic buds. More the production of caulogenic buds proportional to protein expression not even requiring regeneration procedures. With regeneration protocol, WesternHB-55 germplasm can be used for nuclear transformation studies. Also, it can become an effective target for breeding approaches in peanut improvement.

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