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BRIEF COMMUNICATION

Micropropagation in peanut (*Arachis hypogaea* L.)

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Abstract

Multiple shoots in *Arachis hypogaea* L. could be induced from the de-embryonated cotyledons (DC), embryo-axes (EA) and mature whole seeds (MWS) in MS medium supplemented with different levels of benzylaminopurine (BAP). DC was the most suitable explant with 57.9 % induction and more than 40 shoots per explant in 31.6 % of cases. Though EA and MWS had high percent induction at or above 30 mg dm^{-3} BAP, only 10 - 14 shoots per explant were observed. In DC, multiple shoots were confined to the proximal end and in EA they originated from the axillary bud region. Histological studies on DC confirmed the origin of shoots from the region of attachment with the embryo. Shoots could be rooted in MS medium containing 2 g dm^{-3} charcoal and 200 mg dm^{-3} casein hydrolysate. Sixty percent of the rooted plantlets could be established in the field.

Additional key words: embryo-axes, de-embryonated cotyledons, multiple shoots, histology.

Seeds of peanut are a major source of edible oil and protein in India. Peanut has a very limited reproductive efficiency and this comes in the way of generating large segregating populations, which is a prerequisite for breeding programmes, and rapid multiplication of elite mutants. Micropropagation is one of the most exploited techniques in clonal multiplication especially in horticultural crops. This technique has not been fully exploited in peanut probably because of the lack of suitable protocols for high frequency multiplication. A high frequency micropropagation system from DC of mature seeds through multiple shoot regeneration, its ontogeny and the factors influencing multiple shot formation has been reported here.

Surface sterilised mature whole seeds (MWS), de-embryonated cotyledons (DC), and embryo-axes (EA) of *Arachis hypogaea* L. cultivar J11 were used as explants. DC and EA were dissected out of mature seeds. The Murashige and Skoog (1962, MS) medium with the vitamins of B5 medium (Gamborg *et al* 1968), supplemented with BAP (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg dm^{-3}) was used. Explants were cultured in test tubes containing 10 cm^3 of medium in two replicates of 50 tubes each and were incubated at 16-h photoperiod, irradiance of 88 μmol m^{-2} s^{-1} and temperature of 26 ± 1 °C. For detailed observations on the frequency and type of shoots per explant, the experiment was repeated with DC only. Observations were recorded after 15 d of incubation. To confirm the region of origin of multiple shoots, the DCs were cut transversely into two portions which were cultured separately. The decapitated embryo axes (the apical region sliced off) were also cultured. For histological studies, multiple shoot forming explants were fixed in formalin acetic acid (FAA) on the 10^{th}, 15^{th} and the 20^{th} day from the establishment of culture. Paraffin embedding was used for preparation of microtome sections and the sections were double stained with safranine and fast green.

The embryo axes and DC enlarged in size and turned green progressively in culture. In medium supplemented up to 20 mg dm^{-3} BAP, the EA developed into seedling and at 25 mg dm^{-3} BAP and above, the multiple shoots arose from both shoot apices as well as axillary buds (Table 1). The primary root portion below the mesocotyl did not produce any shoots or buds, but produced slight callus in media containing lower concentrations of BAP. Though 100 % induction of multiple shoots was observed in medium with 30 mg dm^{-3} BAP and above, the

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Abbreviations: BAP - 6-benzylaminopurine; DC - de-embryonated cotyledons; EA - embryo-axes; MWS - mature whole seeds.
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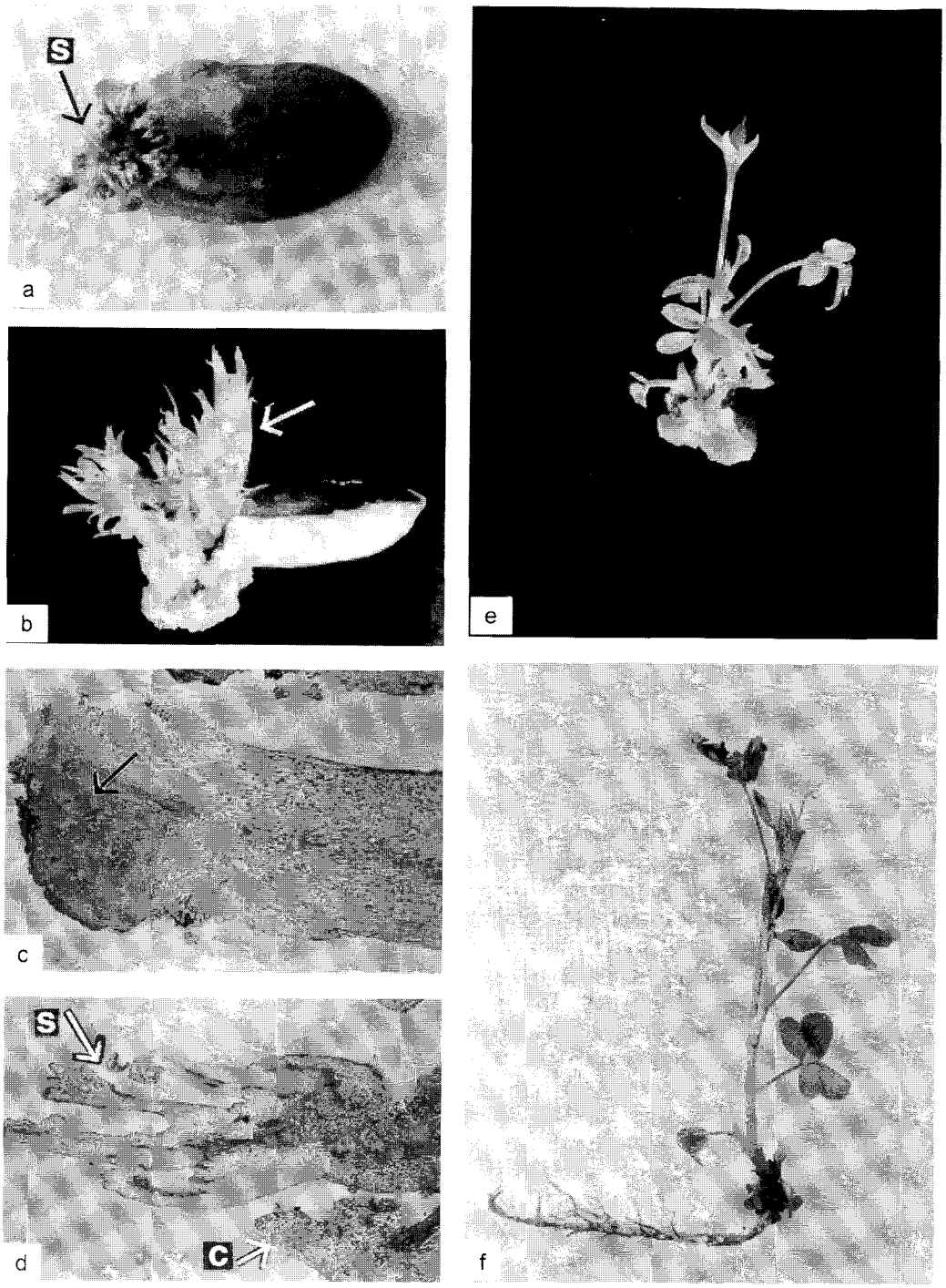

Fig. 1. Developmental stages in the multiple shoot regeneration in peanut:
a - de-embryonated cotyledon (15 d of culture) showing emerging shoots (s - shoots)
b - de-embryonated cotyledon (20 d of culture) showing elongated shoots (*arrow*)
c - longitudinal section of de-embryonated cotyledon (10 d of culture) showing actively dividing cells at the region of attachment of the embryo (*arrow* indicates the zone of division)
d - longitudinal section of de-embryonated cotyledon (20 d of culture) showing elongated shoots (c - cotyledon)
e - shoots separated from explant
f - rooted individual shoot

maximum number of shoots per explant was only 10 - 14. Multiple shoot formation from mature whole seeds had similar frequencies as that of EA. On dissecting the multiple shoots forming seeds, it was observed that the shoots were originating from the embryo apex.

Table 1. Multiple shoot formation [%] in the explants (DE - de-embryonated cotyledons, EA - embryo axes, MWS - mature whole seeds) of peanut cv. J11 at different concentration of BAP [mg dm^{-3}].

BAP	DC	EA	MWS
5	42.11	-	-
10	42.00	-	-
15	57.00	-	-
20	27.62	-	20.00
25	22.00	18.00	22.00
30	14.00	100.00	45.00
35	11.40	100.00	60.00
40	09.27	100.00	74.00
45	12.00	100.00	76.00
50	6.10	100.00	82.00

In de-embryonated cotyledons, multiple shoots were produced from the portion where the cotyledons were attached to the embryo (Fig. 1*a*). Very rarely, a cluster of tiny shoot buds arose from the tip of a small beak growing out from the proximal end (the portion where the cotyledons were attached to the embryo) of de-embryonated cotyledons. Initiation of multiple shoots started at a very low concentration (5 mg dm^{-3}) of BAP in DC when compared to EA (25 mg dm^{-3}). Further, 100 % multiple shoot induction was observed in EA whereas DC had a maximum of 57.9 %. The number of shoots per explant was considerably higher in DC than in EA. Though Illingworth (1968) first reported shoot bud regeneration from de-embryonated cotyledons, the frequency of the induction was not worked out.

The maximum percentage of multiple shoots forming explants (57.9 %) was observed in the medium containing 15 mg dm^{-3} of BAP (Table 2). This was followed by 50 and 42.1 % in medium with 10 and 5 mg dm^{-3} BAP, respectively. Concentrations higher than 30 mg dm^{-3} BAP had a negative effect. The reduction in the frequency of regeneration in DC at BAP concentrations higher than 15 mg dm^{-3} may be due to the increased imbalance among endogenous levels of other growth regulators in the explant. Similar variation in the endogenous growth regulator levels resulting from exogenous supply of cytokinin was reported earlier in somatic embryogenesis of groundnut (Murthy *et al.* 1995). In the other explants, EA and MWS, both of which had regeneration from embryo, such an effect was not observed. The role of the physiological status of the explant in the regeneration response also is well-established (Yeoman and Forche 1980, Radhakrishnan 1996). The number of shoots produced per explant varied with the concentration of BAP in the medium. With 15 mg dm^{-3} BAP, nearly 32 % of the explants could be grouped into category D (>40 shoots per explants) (Table 2). However, it is not very advantageous to have a very high number of shoots per explant, as it makes the subculturing of the tiny shoots difficult and thus affect the recovery of the shoots. The optimum concentration of BAP for multiple shoot induction in our study was 15 mg dm^{-3} as compared to 1 mg dm^{-3} reported by Mhatre *et al.* (1985) and Bhatia *et al.* (1985). Although, Mhatre *et al.* (1985) could induce high percentage of multiple shoots, the numbers of shoots per explant were only 10 to 12. Sastri *et al.* (1992), on the other hand, reported that 25 mg dm^{-3} BAP + 2 mg dm^{-3} NAA could induce maximum multiple shoots in DC.

Table 2. Frequencies (in percentages of the responding explants) of the four different classes of multiple shoot formation (based on number of multiple shoots formed) in de-embryonated cotyledons of the peanut at different concentration of BAP [mg dm^{-3}].

BAP	<10	10 - 25	25 - 40	>40	Total
5	21.05	5.26	15.79	-	42.11
10	15.00	5.00	20.00	10.00	50.00
15	15.79	5.26	5.26	31.58	57.89
20	-	14.29	-	14.29	28.57
25	-	30.00	-	-	30.00
30	-	5.00	10.00	5.00	20.00
35	-	10.00	-	-	10.00
40	-	5.00	-	-	5.00
45	5.0	-	-	5.00	10.00
50	-	5.00	10.00	5.00	20.00

From the DC without their proximal end, no shoot initials were produced revealing their incapability to produce multiple shoots at any level of BAP tried. Bhatia *et al.* (1985) also reported that only proximal ends of cotyledons were capable of producing multiple shoots and development of a beak from this region was essential for the production of shoot buds (Fig 1*b*). Histological studies on the multiple shoot forming explants support this view as the shoot bud originate from the region of attachment by a series of divisions in the initial cells leading to the organogenesis (Figs. 1*c,d*). These meristematic initials are from the axillary bud region of the EA, from where the cotyledons are physically detached and are not *de novo* in origin. In EA also the shoot buds were produced mainly from the axillary bud region though less in number. However, in the further studies with the decapitated embryo axes established that the apical dominance resulted in lesser number of shoots in EA. In most of the cases multiple shoots were induced directly from DC without producing any beak, thus rules out the earlier reports (Bhatia *et al.* 1985) that the beak is an

essential development in multiple shoot formation.

For rooting, the bunches of multiple shoots of about 1 cm long were subcultured on MS basal medium. When these shoots reached the size of about 5 cm (Fig. 1*e*) the individual shoots were separated and cultured on MS medium containing 0.2 % charcoal and 200 mg dm^{-3} casein hydrolysate. Roots were induced the basal part of the 10 - 13 cm long shoots after 4 - 6 weeks under culture (Fig. 1*f*). These plantlets were grown in Hoagland's nutrient solution in a growth chamber at temperature of 30 °C, irradiance of 195 μmol m^{-2} s^{-1}, and relative humidity 80 % for about a week for hardening. Then they were transplanted to field and shaded with wheat straw. When the transplantation was done in the rainy season, about 60 % of these plantlets were established in the field. However, transplantation during the summer season resulted in a higher rate of mortality, reducing the field establishment to 30 - 45 % of the total plantlets transplanted.

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