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HIGHLY EFFICIENT *IN VITRO* ROOT INDUCTION IN PEANUT BY MECHANICAL STRESS METHOD

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ABSTRACT

Peanut (*Arachis hypogaea* L.) belongs to family leguminosae and is one of the world's largest oilseed crops. Efficient tissue culture system is a pre-requisite for improvement of the crop through genetic engineering. Longitudinally halved cotyledons with removed plumule and radical were employed as explants to regenerate shoots in a previously determined best hormone combination. Shoots were inoculated singly or in the form of bunch on two different media in petri plates as well in jars. Shoot bunches in petri plates showed exceptionally high rooting and net survival efficiency on both media compared to all other combinations. Mechanical pressure exerted by low space in petri plate is the most probable reason for development of strong and extensive root system.

Key words: *Arachis hypogaea*, cotyledon, *in vitro* regeneration, mechanical stress, root induction efficiency.

INTRODUCTION

The oil seed crop *Arachis hypogea* L. (groundnut/peanut) belongs to family leguminosae and sub family Papillionacea. It contains 50% oil contents and the remaining portion having 25-30% proteins can be used as meal for food and feed (Ahmed *et al*., 2007). In Pakistan, about 85% of total groundnut is grown in Potohar tract of Punjab, 13% in Khyber Pakhtunkhwa and 2% in Sindh (Govt. of Pak. 2008). Groundnut yield reduces from 20 to 80% due to weed competition with the major loss at early stages of crop development (Royal *et al*. 1997; Bakir, 2011).Most of weeds have broad leaves like groundnut itself so application of broad leave herbicides becomes impossible. Therefore it is necessary to incorporate herbicide resistance genes in groundnut so that herbicide can be applied safely. There is also lack of host resistance against early leaf spot disease commonly called as Tikka disease due to *Fusarium* spp. These are two important constraints (leaf spot disease and weeds) to increase yield of groundnut in the Potohar region of northern Punjab, Pakistan. The introduction of foreign genes from broad resistant sources is possible through genetic transformation. Extensive research has been done in recent years to develop the methods for *in vitro* regeneration and embryogenesis of peanut crop using various explants (cotyledonary nodes, deembryonated cotyledons) and media combinations in different countries (Little *et al*. 2000; Radhakrishnan *et al*. 2001, Tiwari and Tuli 2008). Recently Tiwari and Tuli (2009) developed a protocol using leaflet explants of peanut and got 80% shoot regeneration efficiency. However, in Pakistan the peanut crop remained neglected and there is no prior report for its genetic improvement through innovative approaches. The primary objective of present study was to evaluate the effect of shoots type (bunch vs single shoot), medium (RIM vs SIM) and container (petri plate with less space to exert mechanical pressure vs jar with wider space having no pressure) on root induction.

MATERIALS AND METHODS

Plant Material: The mature seeds of Golden variety of peanut were obtained from Barani Agriculture Research Institute (BARI) Chakwal, Pakistan. Seeds were removed from mature pods and sterilized by soaking into 70 % (v/v) ethanol for one minute followed by the treatment with 20% commercial bleach (colorax) for 10 minutes. Then several washing were made with double distilled autoclaved water and soaked for 3 hour in double distilled water at room temperature. The seed coat and embryos were removed surgically, the cotyledon were cut vertically into two halves to obtain the four cotyledonary explants per seed.

The shoot induction medium (SIM) consisted of MS basal salts, B5 vitamins, 30g/L sucrose and was supplemented with 4mg/L BAP and 0.1mg/L NAA, the best hormone combination proved in our previous experiment (Nazir *et al.,* 2011). For solidification 8g/L agar was added after adjusting the pH at 5.8 at 25°C. After autoclaving, medium was poured into petri plates and five explants were placed with their cut edges in contact with medium in each petri plate for 3 weeks at 25± 2 °C with a photoperiod of 16/8 hours. (The root induction medium (RIM) used in next step of this experiment consisted of half MS basal salts, B5 vitamins, 15g/L sucrose and was supplemented with 2mg/L NAA). After induction of shoots, the explants were divided into

eight equal parts and shifted to fresh medium (RIM or SIM) with density of five explants/container (petri plate or jar).

1. 1/8 of total explants were shifted to petri plates containing RIM after cutting the bunch into single shoots (T_1).
2. 1/8 of total explants were shifted to jar containing RIM after cutting the bunch into single shoots (T_2).
3. 1/8 of total explants were shifted to petri plates containing SIM after cutting the bunch into single shoots (T_3).
4. 1/8 of total explants were shifted to jar containing SIM after cutting the bunch into single shoots (T_4).
5. 1/8 of total explants with bunch of shoots were shifted to petri plates containing RIM (T_5).
6. 1/8 of total explants with bunch of shoots were shifted to jar containing RIM (T_6).
7. 1/8 of total explants with bunch of shoots were shifted to petri plates containing SIM (T_7).
8. 1/8 of total explants with bunch of shoots were shifted to jar containing SIM (T_8).

The experiment was conducted in three factor factorial completely randomized design and was repeated in three batches, each batch having 50 explants.

The rooted plants were transplanted in pots containing peat moss kept in greenhouse. For the first week the pots were covered with polythene sheet to maximise the humidity. The above data for shoot induction and rooting efficiency at different combinations was recorded for each of the four genotypes used in the experiment.

Data analysis: The data collected for various parameters were subjected to analysis of variance and Duncan's Multiple Range Test using M-STATC software (Russell, 1986)

RESULTS AND DISCUSSION

All the three factors have highly significant effect on root induction and survival of rooted plants in pot (Table No. 1). However number of plants survived in pots/total explants inoculated is highly significantly influenced by shoot type (bunch or single shoot) while effect of medium and interaction of both of these is only significant. On the other hand effect of container and all of its interactions are highly significant.

Highest root induction (Table No. 2 and Fig 1-A) was shown either by single cut shoots inoculated on RIM (78.55% in T_3 and 74.95% in T_2) or bunch inoculated in petri plates (73.33% in T7 and 67.88% in T5). On the other hand T_4, T_6 and T_8 showed very poor root induction with values 4.65, 6.64 and 4.94% respectively.

High root induction in T1 and T2 uncovers the fact that RIM causes root induction only from cut shoots, no matter mechanical pressure is exerted or not, while root induction in uncut bunch is not affected by medium but strongly influenced by mechanical pressure showing considerably high root induction in petri plates compared to very low in jars (Fig 2-A,B). Although cut shoots on RIM show better root induction but survival of these plants (Table No. 2 and Fig 1-B) after transplanting to pots is extremely low (8.58% in T2 and 11.38% in T3) as compared to those obtained from bunches (72.55 in T7 and 79.63 in T5). The most rational and logical way to decide the overall efficiency of any method is to look at net result, that is, number of plants survived till maturity in pots per explant inoculated (Table No. 2 and Fig 1-C) . T5 showing 54.22% efficiency is at the top of list closely followed by T7, giving 52.85% results. T1 and T2 which seemed to be the best treatments for root induction (Fig 1-A) proved to be extremely less efficient (8.03 and 6.73 % respectively) in net result, that is, number of plants survived in pots per explant inoculated.

In petri plate the space is limited as compared to that in a jar resulting in mechanical stress on plants which induces rooting process. This method is of immense importance especially in legumes which are thought to be recalcitrant for viable root induction (Rizvi and Sigh 2000; Polock *et al*. 2004). Rooting problem in legumes has prompted many researchers to opt grafting procedure which too is not much successful (Krishnamurthy *et al.* 2000; Sarmah *et al*. 2004; Senthil *et al.* 2004; Sanyal *et al.* 2005; Chakraborti *et al.* 2006). Root induction by mechanical stress is cost effective, quick, one step and simple method resulting in very high success when transplanted to pots.

Peanut plant normally has a very extensive and strong tap root system which is vital for it survival and growth in soil. Plants derived from single cut shoots have very small and delicate root system which is not sufficient to support normal growth. On the other hand roots developed in bunches under mechanical pressure in petri plates are nature identical, dense and strong enough to withstand a new soil environment. Moreover, mechanical pressure in less spacious petri plates hardens the root system just like in situ conditions where soil offers significant resistance in root penetration, hence acclimatising the roots for upcoming constraints.

Root induction in peanut by inoculating single cut shoots in medium containing NAA has been reported by many researchers like Swathi *et al*.(2006); Sharma and Anjajah.(2000); Tiwari and Tuli. (2008); and Tiwari and Tuli. (2009) but the method used in present study is a novel one with no precedence found in literature.

Table No.1. ANOVA for different parameters of plant growth

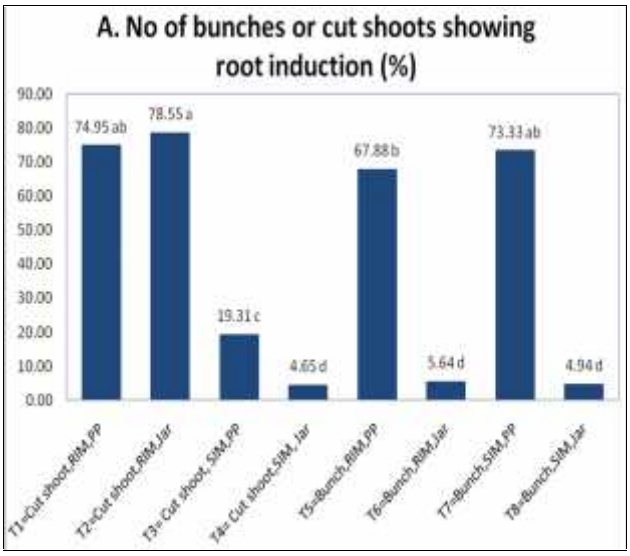
		Number of bunches or cut shoots showing root induction (%)	Number of plants survived in pots/rooted plant (%)	Number of plants survived in pots/total explants inoculated (%)
SOV	**DF**	**Mean square**	**Mean square**	**Mean square**
Shoot type	1	230.206 **	16799.454**	3283.722**
Medium	1	5944.998 **	8.039**	53.491*
Shoot type x medium	1	6651.675**	4.395**	44.636*
Container	1	7623.040**	2138.915**	4302.207**
Shoot type x container	1	5442.383**	311.256 **	3428.455**
Medium x container	1	203.526**	0.074**	0.017**
Shoot type x medium x container	1	65.703ns	484.831**	6.816**
Error	16	23.711	33.220	9.122**
Total	23			

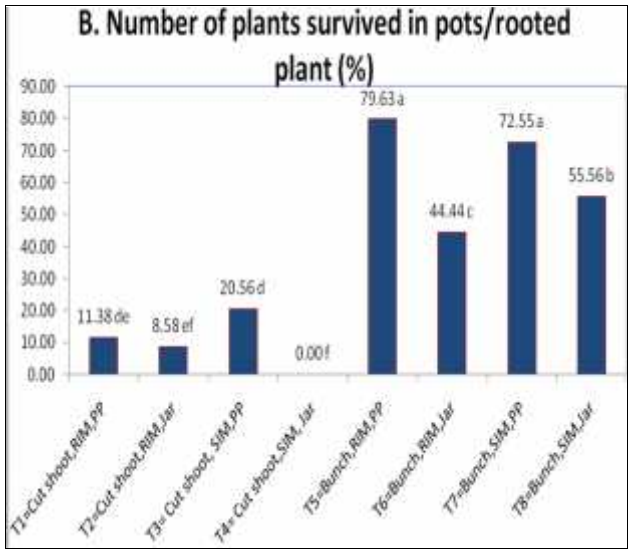
*= significant (P< 0.05); ** (P< 0.01); ns = non significant

Table No. 2. Mean values of different parameters as ranked by Duncun's Multiple Range Test with respect to treatments.

Number of bunches or cut shoots showing root induction (%)		Number of plants survived in pots/rooted plant (%)		Number of plants survived in pots/total explants inoculated (%)	
Treatment No.	**Mean value**	**Treatment No.**	**Mean value**	**Treatment No.**	**Mean value**
T_2	78.55478 ^{a}	T_5	79.62963 ^{a}	T_5	54.22339 ^{a}
T_1	74.94949 ^{ab}	T_7	72.54579 ^{a}	T_7	52.84799 ^{a}
T_7	73.33333 ^{ab}	T_8	55.55556 ^{b}	T_1	8.585859 ^{b}
T_5	67.88145 ^{b}	T_6	44.44444 ^{c}	T_2	6.728272 ^{bc}
T_3	19.30847 ^{c}	T_3	20.55556 ^{d}	T_3	3.883061 ^{bc}
T_6	5.640742 ^{d}	T_1	11.37597 ^{de}	T_8	3.284493 ^{bcd}
T_8	4.94297 ^{d}	T_2	8.576591 ^{ef}	T_6	2.423545 ^{cd}
T_4	4.650325 ^{d}	T_4	0 ^{f}	T_4	0 ^{d}

Values sharing same letters do not differ significantly at 5% level of probability





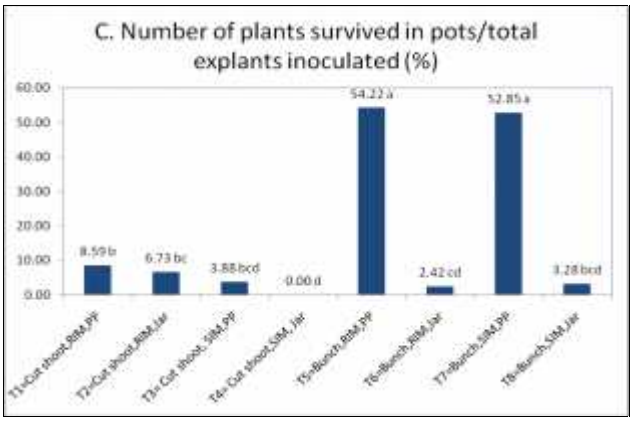
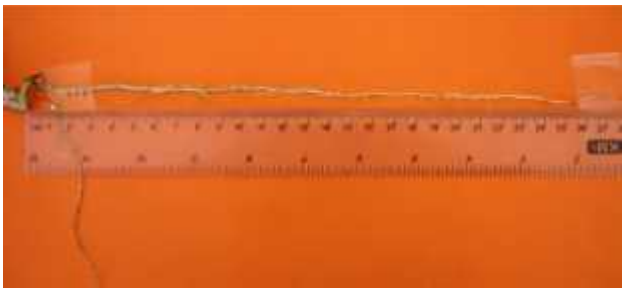


Figure 1.

A. No of bunches or cut shoots showing root induction (%)
B. Number of plants survived in pots/rooted plant (%)
C. Number of plants survived in pots/total explants inoculated (%)

A



B

Figure 2:

A. Rooting from the site of mechanical pressure
B. Some roots grew upto 27 cm

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