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# Standardization of seed biopriming with actinobacteria for seedling vigour in peanut (Arachis hypogaea L.)

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#### Abstrac

**Bio priming:** The interaction of different strains of the same bio agent (organism) with different crop species differ greatly, so screening of different isolates for plant growth promoting traits is a key factor of consideration for successful bio priming. To study the effect of biopriming duration on seed, peanut seeds were bioprimed with actinobacteria for 2, 4 and 6 hr. The seed biopriming of 2hr duration was found to be best as it increased root length, shoot length and dry biomass seedling vigour.

Keywords: Seed bio priming, Seed germination, Rhizobacteria, Actinobacteria, Seed vigour

#### Introduction

Seed is the basic and most critical input for sustainable agriculture. Poor and slower seed germination limits the seedling growth ultimately reducing the crop yield. Efficient seed germination is important for crop yield. Successful establishment of early seedling indeed requires a rapid and uniform emergence and root growth. Development of techniques for fast and homogeneous growth of seeds could be a sustainable approach for better agricultural productivity (Osburn and Schroth, 1988) [13]. In this aspect, improving the quality, germination, and establishment of seed through "seed priming" is a sustainable approach to enhance yields and performance of plants (McDonald, 2000) [12]. Interestingly, primed seeds have been demonstrated to withstand the numbers of abiotic and biotic stresses leading to enhance seed emergence along with crop productivity.

The seed priming is one of the seed quality enhancement techniques where seeds are hydrated to allow metabolic process of germination to take place but preventing radicle protrusion. Seed priming is soaking the seeds in any solution containing our required priming agent followed by redrying the seeds which result into start of germination process except the radicle emergence (McDonald 1999) [11]. There are various techniques which are used for seed priming i.e. hydropriming, halopriming, osmopriming, hormonal priming and bio priming. Hydration using any biological compound is termed as biopriming (Ashraf and Foolad, 2005) [2]. PGPR application through seed priming, soaking the seeds for premeasured time in liquid bacterial suspension, starts the physiological processes inside the seed while radicle and plumule emergence is prevented (Anitha *et al.* 2013) [1] until the seed is sown.

Different priming techniques such as hydro-priming, osmo-priming, solid matrix priming (SMP), nutria-priming (Majda *et al.*, 2019) <sup>[10]</sup>, chemo-priming, thermo-priming, and bio-priming (Panuccio *et al.*, 2018) <sup>[14]</sup> are being used in order to improve seed characteristics, plant productivity as well as alleviate many environmental stresses (Paparella *et al.*, 2015) <sup>[15]</sup> Seed priming with living bacterial inoculum is termed as biopriming that involves the application of plant growth promoting rhizobacteria. This type of priming increases germination rate, seedling vigour and also protects seeds against various soil and seed borne pathogens. Bio priming also enhances the ability of plants to withstand against extreme environmental conditions. The bacteria used in biopriming are able to colonize the rhizosphere and help plant through direct or indirect mechanism.

Biopriming has emerged as an effective approach for increasing seed vigour. There is no standard procedure in biopriming as the treatment duration, concentration depends on species, cultivars and seed types. The optimum such variability is a major limitation of the priming method since numerous trials are required to identify the most appropriate strategy for each situation. There is no general rule concerning modalities of seed priming and there is no clear trend of priming response according to the taxonomic position of the species. This undoubtedly limits the commercial implementation of priming treatments.

Corresponding Author: Suryawanshi Madhuri Pralhad Department of Biotechnology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India Various reports show beneficial effect of seed priming via uniform or early germination, increased nutrient extraction, reduced seed dormancy, etc (Taylor and Harman, 1990; Hill *et al.*, 2008; Farooq *et al.*, 2009, 2019) [17, 7, 6, 5]. According to the published studies, it has been broadly mentioned that priming of seeds mitigates the adverse impact of various biotic (such as, phytopathogens, plant diseases; van Hulten et al., 2006) [18], and abiotic (drought, salinity, flooding) stress factors, that affect the physiology and metabolism of plants via different mechanisms (Kausar and Ashraf, 2003; Basra *et al.*, 2005; Chandra Nayaka *et al.*, 2010; Sharma *et al.*, 2014; Kumar *et al.*, 2016) [8, 3, 4, 16].

Bu *et al.* (2019) explained the effects of endophyte *Epichloe sinica* on *Roegneria kamoji* seedlings under artificial drought stress rendered through PEG. The presence of endophyte was observed to enhance the germination potential as well as the rate of seedling emergence for seeds treated with increased concentrations of PEG.

Peanut (*Arachis hypogaea* L.), also called as groundnut, is one of the world's most important legume crop. It is grown as an oilseed, food and cash crop in wide range of environments. The environmental stress affects the seed germination ultimately reducing the yield. So we made an attempt to standardize the treatment duration of actinobacteria on peanut seeds.

# **Material and Methods**

The groundnut pods of cv. JL24 collected from Seed Unit, UAS, Dharwad were used for the study.

#### **Actinobacteria Strains**

The actinobacteria isolates were collected from culture collection at Department of Biotechnology, College of Agriculture, UAS, Dharwad.

# **Seed Bio Priming**

The peanut seeds were surface sterilized with sodium hypochlorite (1 %) for five minutes and washed thrice with sterile water. The actinobacteria culture was grown in tryptic soya broth for 5days was used for biopriming. The seeds were bio primed with actinobacteria culture (CFU 10<sup>8</sup>) by soaking them for 2, 4 and 6 hr and then shade dried for 24 hours.

# **Seed Germination**

Germination test was conducted in four replications each of 100 seeds as per the ISTA procedure (Anon., 2014) by adopting between paper method in the walk in seed germinator room maintained at  $25 \pm 2^{\circ}$ C temperature and 90  $\pm$  5 per cent relative humidity. On tenth day of germination test the final germination count based on normal seedlings was made and expressed as germination percentage.

#### **Root Length**

The root length was measured from the collar region to the tip of the primary root. The mean root length was expressed in centimetres (cm).

# **Shoot Length**

The shoot length was measured from the collar region to the tip of primary leaf. The mean shoot length was worked out and expressed in centimetres (cm).

## Seedling Vigour Index (SVI)

Vigour index was computed by using the following formula and expressed in number (Abdul-Baki and Anderson, 1973). Seedling vigour index = Germination % × [Shoot length + Root length].

# **Root and Shoot Dry Weight**

The seedlings earlier used for measuring of root and shoot length were also used to determine the seedling dry weight. The seedlings were kept in butter paper bag and dried in a hot air oven maintaining at 80 °C for 48 h and cooled in a desiccator for 60 minutes. The weight of dried seedlings was and expressed in grams.

### **Results and Discussion**

Improving the plant performance and productivity under normal and stressed environmental conditions is of prime importance for sustainable agriculture. The efficiency of priming is influenced by a number of factors such as ventilation, light, temperature, time and seed quality. The priming with actinobacteria culture had significant influence on the peanut seeds. It was observed that the increased duration of actinobacteria treatment had the negative effect on seedlings. The duration of treatment affected the root and shoot length, dry biomass and seedling vigour index (Table 1.). The root length in the 2 hr treatment with actinobacteria was significantly more than 4 and 6 hr treatment. The root length observed was 25.1, 27.9, 29.2 and 28.3 cm in isolate AUUB54, AUUB117, AUDT559 and AUDT643 respectively. Root length was less in 4 and 6 hr treatment than control unprimed seedlings. The shoot length in the 2 hr treatment with actinobacteria was found to be highest followed by 4 hr treatment. The shoot length observed was 26.3, 22.4, 23.8 and 23.2 cm in isolate AUUB54, AUUB117, AUDT559 and AUD4T643 respectively. The germination percentage increased many folds compared to control may be due to the production of some plant growth promoting compounds by the Actinobacteria. The vigour index was highest in the 2 hr of actinobacteria culture priming followed by 4 hr. The seedling vigour reduced at 6 hr treatment as compared to unprimed treatment. The vigour index for 2 hr actinobacteria isolates AUUB54, AUUB117, AUDT559 and AUDT643 was 4523.0, 4586.0, 4770 and 4635.0 respectively. The isolates AUUB54, AUUB117 and AUDT559 showed significant increase in root dry weight in the 2 hr treatment with actinobacteria than 4 and 6 hr treatment. The root dry weight observed was 0.082, 0.085, 0.089 and 0.078 g in isolate AUUB54, AUUB117, AUDT559 and AUDT643 respectively. The shoot dry weight observed was 0.362, 0.328, 0.372 and 0.334 g in isolate AUUB54, AUUB117, AUDT559 and AUDT643 respectively. The increased dry root and shoot weight demonstrated the biomass accumulation due to treatment. Ramamoorthy et al., (2000) reported that priming of Azospirillum enhanced seedling vigour seedling length and dry weight of high and low vigour seed lots in rice. This study shows that the prolonged treatment duration can have negative impact on seedling. Hence the optimization of treatment duration is of prime importance. The increased seedling vigour can enhance the crop productivity.

Shoot dry Weight Duration Germination **Root Length Shoot Length** Root dry Weight Seedling Vigor **Traits %** (hr) (cm) (cm) (gm) (gm) Isolates 0.069 0.244 2670 Control 60 24.5 20 2 25.1 26.3 0.082 0.41 4523.2 88 4 22.2 25.9 0.067 3799.9 AUUB54 79 0.362 6 74 20.4 21.4 0.066 0.198 3093.2 2 91 27.9 22.5 0.085 0.328 4586.4 4 22.4 AUUB117 86 24 0.074 0.28 3990.4 6 64 20.3 18.6 0.067 0.238 2489.6 2 90 29.2 0.089 0.372 4770 23.8 AUDT559 4 80 28.3 20.6 0.066 0.214 3912 6 80 20 18 0.071 0.244 3040 90 28.3 23.2 0.078 0.334 4635 2 AUDT643 4 70 24.8 20.7 0.066 0.318 3185 23.5 14.9 0.062 0.029 2304 6 60 C.D. 2.13 2.8 0.004 0.038 SE(m) 0.75 0.96 0.001 0.013

**Table 1:** Effect of seed priming with actinobacteria on peanut seedlings

#### **Conflict of Interest**

The authors declare no conflict of interest.

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