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Evaluation of role of testa, cotyledon and embryonic axis in manifestation of seed dormancy in groundnut varieties

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Abstract

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops. The semi-spreading and spreading kinds (Virginia types) have extensive dormancy periods, whereas the majority of Spanish and Valencia bunch cultivars lack seed dormancy. A study was undertaken in the Department of Seed Science and Technology, Tamil Nadu Agricultural University to determine the involvement of the testa, cotyledon and embryonic axis in the manifestation of seed dormancy in two different varieties of groundnut VRI 8 (Non-dormant) and CO 6 (Dormant). A germination test was conducted for the seeds with testa, without testa and embryonic axis by using freshly harvested seeds of variety VRI 8 and CO 6. The groundnut CO 6 dormant variety with testa recorded the lowest germination (17.6 %) and highest fresh ungerminated seeds (76 %) than non-dormant variety VRI 8 (71.2 % & 18.4 %). The highest viable seed was recorded in CO 6 dormant variety (92 %) than VRI 8 (80%) and embryonic axis of both VRI 8 and CO 6 recorded 98.4 % germination. Total phenol content of CO 6 groundnut seed testa was much greater (116.81 µg/g) than non-dormant VRI 8 seed testa (41.8 µg/g). The dormant variety CO 6 contains more phenolic compounds in the seed coat than the cotyledon and embryonic axis.

Keywords: groundnut, testa, cotyledon, embryonic axis, dormancy

Introduction

Groundnut (*Arachis hypogaea* L.) is a leguminous plant often grown in the tropics and subtropics. In 2020-21, India ranks first in groundnut acreage and is the world's second largest producer with the production of 101 lakh tonnes and productivity of 1816 kg per hectare. In terms of growing habit, groundnut cultivars are divided into three categories viz., bunch (Spanish), semi spreading (Virginia bunch), and spreading (Virginia runner). Seed dormancy is the temporary failure of a viable seed to germinate, after a specific length of time and in a particular set of environmental conditions that allow germination after the restrictive state has been terminated by either natural or artificial conditions [1]. Seeds of Spanish and Valencia bunch types of groundnut are usually non dormant, whereas those of Virginia types are dormant and maintains the dormancy even up to two months [2]. However, in the Spanish kind of groundnut, a limited period of dormancy is essential to prevent in situ seed germination in the field due to unseasonal rain at crop maturity. Pre-harvest sprouting in groundnut is undesirable because it results in significant seed loss in both quantity and quality. In situ germination in bunch types has been observed to cause a 20-40 per cent yield reduction and reduces seed quality and storability [3]. The yield losses due to viviparous germination can be avoidable if we have bunch varieties possessing a short period of dormancy (3-4 weeks). In groundnut, seed dormancy has been reported to be controlled by two hormones viz., abscisic acid which inhibits sprouting and ethylene which is accumulated in storage to break dormancy to allow germination [4,5] reported that components of groundnut seed, including the seed coat, cotyledons and embryo, were involved in imposing dormancy. To induce a short period of dormancy in Spanish groundnut and release of dormancy in Virginia types for early germination through breeding programme, seed treatment and foliar spray, study of seed components responsible for dormancy in groundnut is vital.

Materials and Methods

Freshly harvested groundnut seeds of VRI 8 (non-dormant) and CO 6 (dormant) were collected from Regional Research Station, Vridhachalam, Tamil Nadu, India. Pods were shelled to separate the kernels without damage to testa.

To study the role of testa, cotyledon and embryonic axis in manifesting fresh seed dormancy, germination test was conducted for individual seed component. Kernels were soaked for 30 minutes and removed the testa by sharp needle without damaging the kernel. The experiment was conducted by adopting factorial completely randomized block design (FCRD) with four replications. Germination test was conducted and seed quality parameters were observed.

The germination test was conducted with 400 seeds in four replications each with 100 seeds using roll towel method. Seeds with testa and without testa were placed in moist wrapped towels between paper substrates for germination testing. The substrate was wrapped in plastic bags and stacked in baskets. The baskets were kept at a temperature of $25 \pm 1^\circ\text{C}$ and a relative humidity of $95 \pm 2\%$. The germination was calculated using normal seedlings as indicated in ISTA regulations after ten days of incubation [6]. Germinability of embryonic axis was evaluated through in-vitro method *i.e.*, aseptically excised embryonic axis were placed on a sterile, hormone free MS culture medium (Murashige and Skoog). The petri dishes were incubated at $25 \pm 1^\circ\text{C}$ and 18 h light for 10 days. The excised embryonic axes which produced normal radicles of > 5 mm length were considered as germinated.

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Fresh ungerminated seed (%) was assessed after the evaluation of germination (%). The seeds which do not produce seedlings however remain fresh at the end of test period are classified as fresh ungerminated seeds, and the mean is expressed as percentage.

Root length and shoot length (cm) was measured by using ten normal seedlings. Root length was measured from the collar region to the tip of the primary root and measured the shoot length from the collar region to the growing tip of the shoot. Then the mean value was calculated and expressed in cm. Seedling vigour index was calculated as per the method suggested by [7] and the mean values were expressed in whole number.

Seedling vigour index = Germination (%) x Total seedling length (cm)

Tetrazolium viability test was conducted for both varieties, 200 seeds in four replications each with 50 seeds were taken and pre-conditioned in water for 3 hours. Then the seeds were bisected longitudinally into two halves and transferred to beaker containing 0.5 % triphenyl tetrazolium chloride. This was kept for 2 hours in an incubator maintaining 40°C for staining. Then, the stained embryos were grouped into viable and non-viable and expressed as percentage. Total phenol content ($\mu\text{g/g}$) was estimated using protocol proposed by [8]. The results were statistically analysed [9].

Result and Discussion

The germination percentage is an excellent indicator of growth potential and survival of seeds, irrespective of factors responsible for loss of viability [10]. The germination percentage of seed with testa was significantly varied with varieties. The groundnut CO 6 dormant variety with testa recorded the lowest germination (17.6 %) (Table 1) and highest fresh ungerminated seeds (76 %) (Table 2) while non-dormant variety VRI 8 seed with testa recorded the highest germination (71.2 %) (Table 1) and lowest fresh

ungerminated seeds (18.4 %) (Table 2). In contrast, in both the varieties, seeds without testa recorded maximum germination percentage. The germination percentage of seed without testa in dormant variety CO 6 registered 73.6 per cent germination which is 56 % increase over seeds with testa (Fig 2). Seeds without testa in non-dormant variety VRI 8 recorded 77.6 per cent germination which is only 6.4 % higher than the seeds with testa (Fig. 2). The results revealed that removal of outer layer of the seed testa helped in the loss of dormancy in groundnut and increased the germination (%). [11] reported that the site of dormancy in groundnut is the seed coat and higher germination was evident on removal of the entire seed coat. In the present study, seed viability percentage of non-dormant and dormant varieties were assessed. The highest viability percentage was recorded in CO 6 dormant variety (92 %) than VRI 8 (80 %) but CO 6 recorded only 17.6 % germination and 76 % of fresh ungerminated seeds which might be viable but could not be able to germinate due to presence of seed dormancy (Fig. 1).

Embryonic axis of VRI 8 (non-dormant) and CO 6 (dormant) was cultured in MS media and observed 98.4 % germination in embryonic axis of both VRI 8 and CO 6 (Table 1). The results indicated that embryonic axis might not contain any inhibitors responsible for dormancy in CO 6 variety. In CO 6, seeds with testa and without testa recorded maximum root length (19.0 cm, 22.9 cm, respectively), shoot length (10.2 cm, 12.3 cm, respectively) than the seed with testa and without testa in VRI 8 (15.8 cm, 20.0 cm; 8.4 cm and 7.4 cm, respectively) (Table 3). Maximum vigour index was recorded in VRI 8 (1920) than CO 6 (1560) (Table 4).

In the present study, the results indicates that total phenol content of CO 6 groundnut seed with testa was much greater ($116.81 \mu\text{g/g}$) than seeds without testa ($29.5 \mu\text{g/g}$) and embryonic axis alone ($10.0 \mu\text{g/g}$). The total phenol content in non-dormant VRI 8 variety of groundnut seed with testa ($41.8 \mu\text{g/g}$), without testa ($26.18 \mu\text{g/g}$) and embryonic axis alone ($6.17 \mu\text{g/g}$) was very low than the dormant variety (Fig. 3). The results confirmed that mainly the seed coat contributed to the presence of majority of phenolic compounds in dormant variety.

Total phenols distributed in different parts of the seeds (testa, cotyledon and embryonic axis). More amount of phenols are present in the seed testa followed by cotyledon and embryonic axis. Total phenol content in seed coat, raw kernel and cotyledons of six peanut varieties ranged from 97.3 to 133.5, 3.28-9.20 and 0.88-1.85 mg GAE/g, respectively [12]. It indicates that the seed testa has a strong correlation with total phenolic content of kernel. In the present study, maximum phenol content in the seed coat of CO 6 variety might have inhibited the germination even though the seeds showed viability. Phenolic compounds may inhibit seed germination and cause dormancy by inhibiting cell elongation or they may deprive the embryo of oxygen because of consumption of oxygen for their oxidation [13, 14] reported that dormancy in groundnut seed was positively related to phenol content and that seed germination in non-dormant cultivars appeared to be due to faster release of these chemicals from the seed by leaching [15]. Observed continuous increase of some phenolic acids and phenolic compounds during seed development in dormant varieties of groundnut [16]. Studied bioassays of aqueous extracts of lettuce and tomato seed and reported that phenolic acid responsible for dormancy. The results of present study confirmed the report of [17] studied the dormancy in Spanish and Virginia genotypes of groundnut and observed

that the whole seed dormancy was higher in both Spanish and Virginia types as compared to embryo dormancy. The genotype of groundnut Dh-3-30 indicated dormancy period of 20 -30 days with whole seeds but did not show embryo dormancy, revealed the presence of dormancy factors in seed coat.

Conclusion

The maximum germination percentage was observed in dormant groundnut variety CO 6 after removal of testa than the non-dormant variety VRI 8. The reduced germination percentage in dormant variety might be due to presence of phenolic compounds in the seed coat than the cotyledon and embryonic axis.

Table 1: Role of seed components on germination (%) in groundnut varieties

Factors	Variety 1 (VRI 8)	Variety 2 (CO6)	Mean
T1- Seed with testa	71.2	17.6	44.4
T2- Seed without testa	77.6	73.6	75.6
T3- Embryonic axis alone	99.0	99.0	98.4
Mean	82.4	63.2	72.8
	V	T	V X T
SEd	2.03	2.49	3.53
CD (P=0.05)	4.20	5.15	7.29

Table 2: Role of seed components on fresh ungerminated seeds in groundnut varieties

Factors	Fresh ungerminated seeds (%)		
	Variety 1 (VRI 8)	Variety 2 (CO 6)	Mean
T1- Seed with testa	18.4	76.0	47.2
T2- Seed without testa	8.0	9.6	8.8
Mean	13.2	42.8	28.0
	V	T	V X T
SEd	2.38	2.38	3.37
CD (P=0.05)	5.05	5.50	7.14

Table 3: Role of seed components on root length and shoot length in groundnut varieties

Factors	Root length (cm)			Shoot length (cm)		
	Variety 1 (VRI 8)	Variety 2 (CO 6)	Mean	Variety 1 (VRI 8)	Variety 2 (CO 6)	Mean
T1- Seed with testa	15.8	19.0	17.4	8.4	10.2	9.3
T2- Seed without testa	20.0	22.9	21.4	7.4	12.3	9.9
Mean	17.9	21.0	19.4	7.9	11.3	9.6
	V	T	V X T	V	T	V X T
SEd	0.21	0.21	0.30	0.12	0.12	0.17
CD (P=0.05)	0.45	0.45	0.63	0.25	0.25	0.36

Table 4: Role of seed components on vigour index in groundnut varieties

Factors	Variety 1(VRI 8)	Variety 2 (CO 6)	Mean
T1- seed with testa	1711	516	1114
T2- seed without testa	2129	2603	2366
Mean	1920	1560	1740
	V	T	V X T
SEd	88.4	88.4	125
CD (P=0.05)	187.4	187.4	265.0

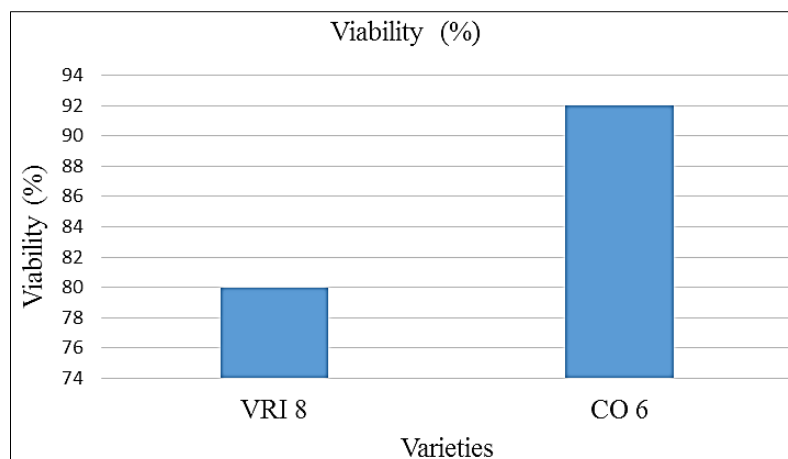


Fig 1: Viability (%) of dormant and non-dormant groundnut varieties

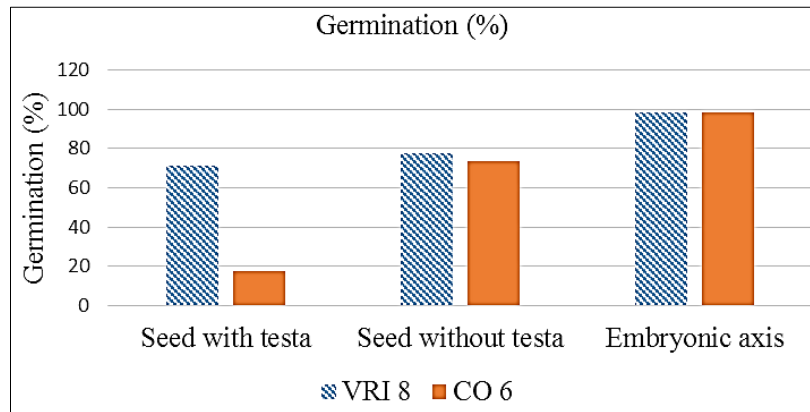


Fig 2: Role of seed components on germination (%) in dormant and non-dormant varieties of groundnut

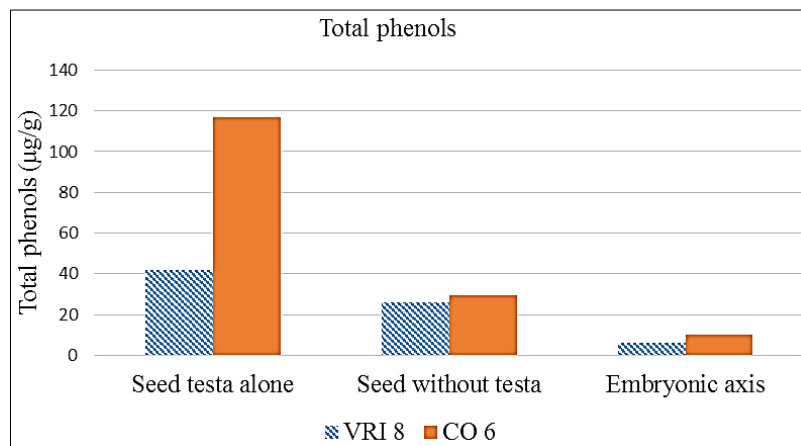


Fig 3: Total phenol content (µg/g) of seed testa, cotyledon and embryonic axis in dormant and non-dormant varieties of groundnut

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