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Physiological and Biochemical Basis of Seed Dormancy in Groundnut (*Arachis hypogaea* L.) Cultivars

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ABSTRACT: The experiment was undertaken at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore to study the physiological and biochemical basis of seed dormancy during seed development and maturation in groundnut. Two groundnut varieties namely dormant variety CO 6 and non-dormant variety VRI 8 were used for this study. The research on The stage of dormancy induction during seed development and maturation revealed that the dormant variety CO 6 confirmed acquisition of dormancy during seed development by registering only 20% germination (20 %) at maximum seed viability (100 %). It recorded 77% fresh ungerminated seeds (FUS) at maturity stage (130 days after sowing). In the nondormant variety VRI 8 percentages of germination, viability and FUS at maturity stage (105 days after sowing) were 78,100 and 20 % respectively. Total phenolic content at maturity stage in seeds of dormant variety CO 6 was significantly higher (77.95 µg/g) over the non-dormant variety VRI 8 (46.62 µg/g). Assessment of hormones at maturity stage revealed that presence of high level of ABA (0.912 µg/g) and low level of GA_3 (1.117 µg/g) in dormant variety CO 6 compared to non-dormant variety VRI 8 (0.043 µg/g and 1.141 µg/g, respectively). Determination of volatile compounds in groundnut varieties confirmed that germination inhibitors such as phenols and decanoic acid were found in the seeds of dormant variety at maturity stage, whereas the seeds of non-dormant variety had germination promoting compounds such as phenyl acetic acid and oleic acid.

Key words: Groundnut, Seed development, dormancy, hormonal regulation, volatile profile

Groundnut (*Arachis hypogaea*L.), belonging to Fabaceae family is the most important oilseed and cash crop in semi-arid tropics [1]. Groundnut (*Arachis hypogaea* L.) has three sub species *viz.*, 1) Spanish (ssp. *fastigata*var. *vulgaris*) 2) Valencia (ssp. *fastigata* var. *fastigata)* and 3) Virginia (ssp. *hypogea* var. *hypogea).* Spanish types are grown predominantly in the semi-arid regions of Asia and Africa which has short growing season and non-dormant seeds. Lack of short term fresh seed dormancy in bunch type genotypes result *in situ* sprouting and it is more problematic in areas where moisture retention capacity of the soil is high [2]. A loss of 20-50 per cent in pod yield in bunch groundnut has been reported due to *in situ* germination. Seeds produced during *rabi* and summer seasons lost about 50% viability within 4-5 months [3] due to increased susceptibility of groundnuts to *Aspergillus* infection, which causes aflatoxin contamination and results in low seed quality.

Seed dormancy is an important agricultural trait, with too little dormancy leading to pre-harvest sprouting or too much dormancy causing inability to germinate the seeds, delayed germination or non-uniform germination, that leads to poor establishment and low yield. In groundnut, seed dormancy has been reported to be controlled by two hormones *viz*., Abscisic acid which inhibits sprouting and ethylene which accumulates in embryonic axis during storage to break dormancy and allow germination [4]. Prolonged seed dormancy is reported in Virginia types, which accounts for about 60% of groundnut production [5] and is an undesirable character. In dormant kinds, the seeds from the *kharif* crop cannot be utilized to raise the *rabi* or summer crop unless there is at least a three-month gap between the *kharif* crop's maturity and the planting time. Hence, the Spanish and Valencia groups need induction of fresh seed dormancy for short period to prevent *in situ* germination and Virginia group need breaking of dormancy to facilitate the use of fresh seeds for sowing in the next season. Development of short duration peanut varieties having fresh seed dormancy to prevent yield losses due to field sprouting in unpredictable rainfall environments is the need of the hour but very little information is available about nature of dormancy, dormancy induction stage and physiological mechanism responsible for dormancy in groundnut. Therefore, clear understanding of physiological and genetic basis of *in*

situ sprouting and seed dormancy at crop maturity is required before launching any breeding program for evolving early Spanish groundnut varieties with desirable fresh seed dormancy. Against these background, a research was conducted to assess the physiological and biochemical basis of seed dormancy in groundnut for exploitation in breeding programme for development of desirable traits.

MATERIALS AND METHODS

The investigation was carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during rabi 2020-21. Groundnut non dormant variety VRI 8 obtained from the Regional Research Station (RRS), Viridhachalam and dormant variety CO6 received from the Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore were used for the research.

The groundnut non dormant variety VRI 8 and dormant CO 6 were sown in 25 cents with a spacing of 30 x 10 cm in 4×4 m plot size each. The seed rate compensation was done following Manjunathaswamy and Narayanaswamyb [6] to maintain the plant population and prescribed crop management practices were followed. The plants were observed daily for flower initiation and tagged at 50% of flowering (approximately 250 plants per plot) for documenting physiological and biochemical properties during seed development and maturation. Twenty plants at each time were uprooted randomly at five days interval by using 50% flowering as the indicator from 50 days after sowing (DAS) until 120 DAS in non dormant variety VRI 8 and from 65 DAS to 140 DAS in dormant variety CO6. At each harvest, pods with uniform size were selected for analyzing physiological and biochemical properties of developing seeds.

Seed germination test

The germination test was carried out with four replicates of hundred seeds each using sand in a germination room maintained at 25 ± 2 °C temperature and 95 ± 3 per cent RH %, illuminated with fluorescent light. After the test period of ten days, the seedlings were evaluated. The mean germination was determined and reported as a percentage based on the mean number of normal seedlings produced.

Tetrazolium viability test

Tetrazolium viability test was conducted at different seed development stages, 200 seeds in four replications each with 50 seeds were taken and preconditioned in water for 3 hours. Hydrated seeds were bisected longitudinally into two halves and transferred to beaker containing 0.1% triphenyl tetrazolium chloride. This was incubated for 2 hours at 40° C for staining. Based on the staining pattern, the seeds were grouped into viable and non-viable and expressed in percentage.

Fresh ungerminated seeds

The germination test was carried out in accordance with ISTA [7] and the seeds which do not produce seedlings however remain fresh at the end of the prescribed period were categorized as fresh ungerminated seeds, and the mean expressed as percentage.

Total phenols (µg/g)

Total phenolic content of the seed in different developmental stages was calculated using protocol proposed by Singh *et al.* [8].The standard stock solution was prepared by using gallic acid. 1g of seed sample was taken and homogenized using 10 ml of 80 percent ethanol and samples were centrifuged at 3000 rpm for 10 minutes. The 1 ml of aqueous solution was taken in test tube and 0.5 ml of folin ciocalteau reagent and 2ml of 20 percent sodium carbonate were added and it was kept in a water bath for 10 minutes. The tubes were covered with aluminium foil and incubated for 30 minutes at room temperature. The absorbance was taken at 660 nm.

Hormone analysis through HPLC

Reverse phase HPLC was used to analyse GA_3 and ABA found in VRI 8 and CO 6 varieties. The binary solvent system used water with 0.1% formic acid (vol/vol) (A) and mobile phase (B).YMC C18 column were used for analysis. The column dimension was 100 x 2 mm and I.D. 1.9 µm. Solvent flow rate was 0.3ml/min. Column oven temperature was 30° C and detection limit for ABA was 260 nm and GA3 was 345.1nm. Retention time was 3.05 min for GA_3 and 2.33 min for ABA and linearity of standard calibration curve was 99.99%. Seed samples were frozen with liquid nitrogen and 10 ml extraction solvent, containing 2-propanol/H O/HCL (2:1:0.02.v/v/v) and 20 ml of dichloromethane was added, and kept on a shaker at a speed of 100 rpm.for 30 min at 4° C, then centrifuged at 13000 rpm for 5 min at 4° C. Collected 2.0 ml of the solvent from the lower phase and injected 50 µl of sample solution into the reverse- phase C18 HPLC column for hormone analysis.

Secondary metabolites through GCMS

The air sample was taken from the fresh seeds of dormant and non-dormant varietiesusing 20 ml gas tight syringeand subjected for GC-MS analysis. While taking the sample the needle of the syringe was inserted just nearing the surface of the seed layer. Then the air sample was transferred to headspace vial and directly injected to GC-MS (Thermo Scientific Trace GC Ultra chromatograph system (Thermo Fisher Scientific, Austria), coupled to thermo scientific DSQ II quadruple mass spectrometer). The Helium (99.9%) gas was used as a carrier gas with flow rate of 1.0 ml/min and pressure of 60-100 Psi, 400-700 Kpa. Volatile compounds from air sample were separated by phenyl methyl silicon fused-silica capillary column (TG-5 MS, 30m in length, 0.25µm and 0.25µm film thicknesses). The column was initially held at 40ºC for 1 min, and then it was increased at 5ºC/min to reach 200ºC and at 10ºC/min to reach 260ºC withhold time of 5-6 min, respectively. Injection volume of 2ml was taken in split less mode. The injector and detector were constantly maintained at temperature of 260ºC and 270ºC respectively with a total run time of 45 min for good separation of the diverse compounds. Volatile compounds were identified by the fragmentation pattern of individual compound and confirmed with the NIST (National Institute of Standards Library database).

Data analysis

The data obtained from the experiment were analysed using SPSS software and data were statistically analysed using analysis of variance (ANOVA) in Excel. The treatment means were analyzed by Duncan's multiple range test (DMRT) at $p < 0.05$.

RESULTS AND DISCUSSION

In this study the changes in reproductive development of groundnut CO 6 dormant variety was examined to assess the stage of dormancy induction in comparison with VRI 8 non-dormant groundnut variety.

Physiological changes in dormant and non-dormant varieties

The tetrazolium test revealed that seed viability was 48 per cent in dormant variety CO 6 at 95 DAS and it was increased to 100 per cent at 140 DAS. In non dormant variety VRI 8, the seed viability was observed as 56 per cent at 85 DAS and it was increased to 100 per cent at 120 DAS (Fig.1). In CO 6, the seed germination initiated at 95 DAS (12 %) and reached only 20 per cent at 130 DAS and thereafter germination was reduced to 16 per cent at 140 DAS (Plate 1). In VRI 8, the germination was observed at 85 DAS (36 %), then increased gradually to 78 per cent at 105 DAS, after that the germination was reduced to 75 per cent at 120 DAS (Fig.2). In CO 6, the fresh ungerminated seed was increased from 35 percent at 95 DAS to maximum of 77 per cent at 130 DAS. In VRI 8, the fresh ungerminated seed was increased from 14 per cent at 85 DAS to 18 per cent at 105 DAS

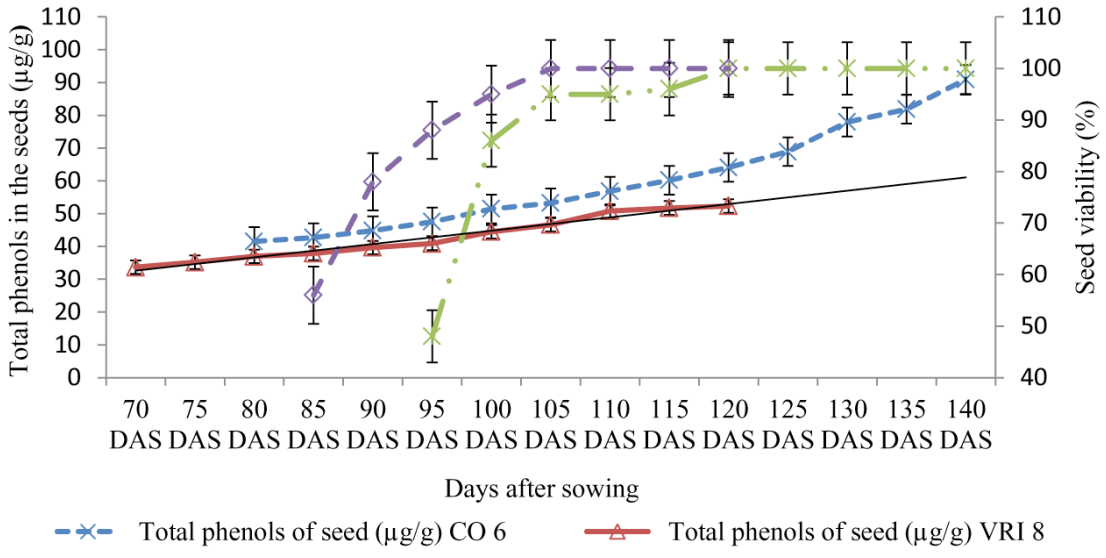

Figure 1. Relationship between seed developmental stages on seed viability (%) and total phenol (µg/g) in seeds of groundnut varieties CO 6 and VRI 8

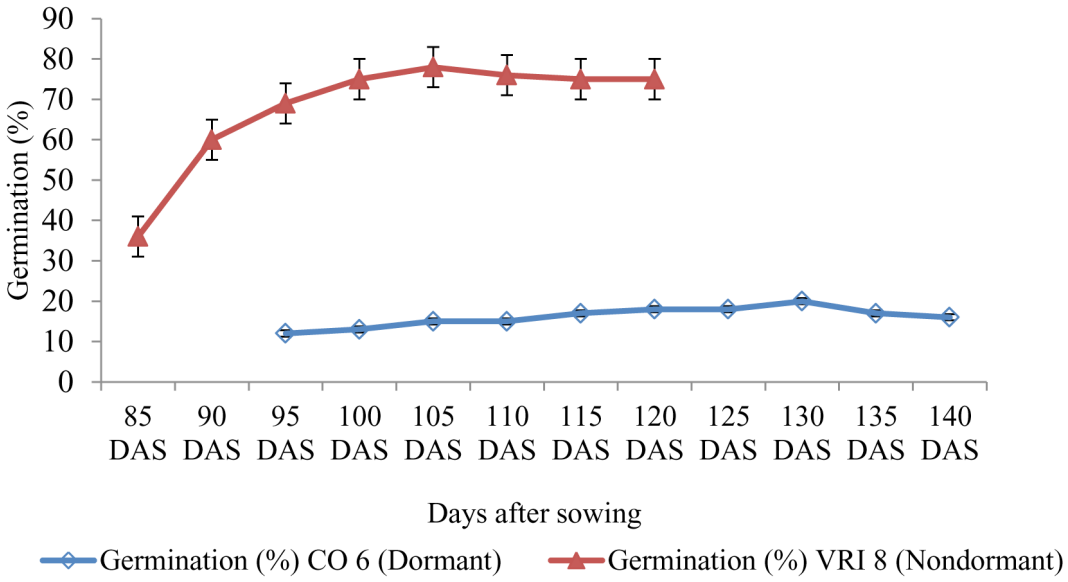

Figure 2. Seed developmental stages on germination (%) in groundnut varieties CO 6 and VRI 8

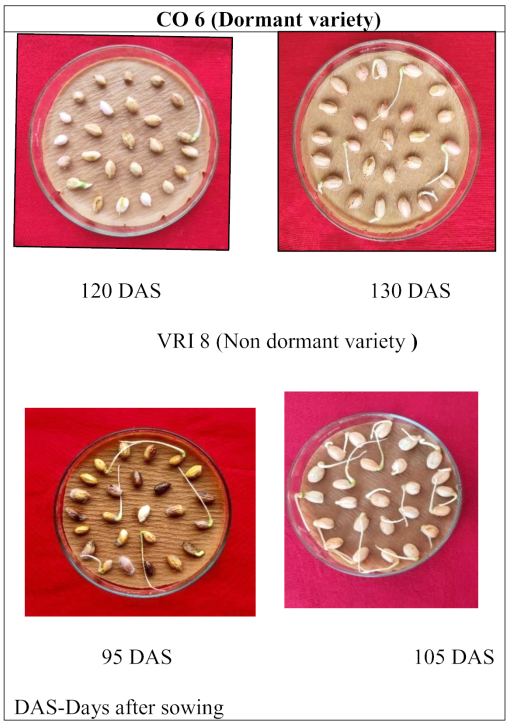

Plate 1. Variation in germination (%) in groundnut dormant variety CO6 and Non dormant variety VRI8

and then decreased to 15 per cent at 120 DAS (Fig.3).

In groundnut dormant variety CO 6, 48 per cent of seeds were viable at 95 DAS and showed 100% viability at 120 DAS. However, the ability to germinate was significantly lower at 95 DAS compared with 120 DAS. This was due to the underdeveloped embryo and cotyledons at 95 DAS and reduction in germination of groundnut at maturity was probably due to presence of dormancy. The percentage of fresh ungerminated seed in the CO 6 dormant variety was greater during maturation whereas in non-dormant variety, presence of fresh ungerminated seeds was lesser. High viability from 120 DAS onwards with relatively low germination and high fresh ungerminated seeds is due to seed dormancy, which prevents *in situ* germination [9]. Seed dormancy was imposed gradually during seed maturation and was higher during physiological maturity for groundnut resulting in a drastic reduction in germination percentage [10].

The non-dormant variety (VRI 8) started to germinate at 85 days after sowing and at this phase, the germination was only 36 percent. This might be due immature seeds with a greater moisture content. Thereafter, it progressively increased until it reached a maximum of 78 percent at 105 DAS. This is due to the fact that the percentage of mature seeds increased steadily coupled with rapid increase in seed moisture content. In dormant variety (CO 6), germination of 12 per cent at 95 DAS was noticed and this might be because of seeds harvested at early stage are immature and underdeveloped, resulting in poor germination compared to seeds collected at physiological maturity. There after the germination gradually increased up to only 20 per cent at 130 DAS and dormancy was found to be

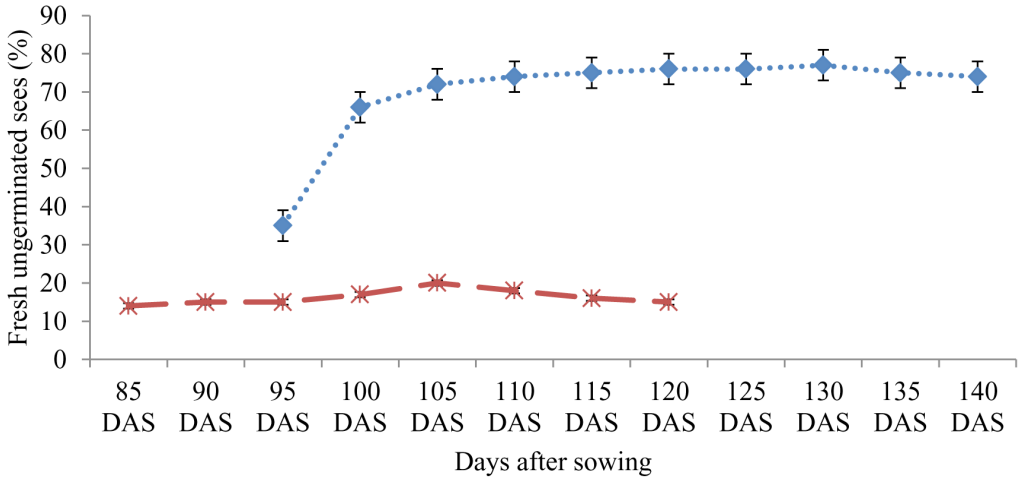

Figure 3. Influence of seed developmental stages on fresh ungerminated seeds (%) in groundnut varieties CO 6 and VRI 8

significantly higher at physiological maturity stage. This result agreed with the findings of earlier researchers, who found that the highest seed germinability was not always at physiological maturity [11]. The reduction in germination of groundnut at maturity was probably due to dormancy that prevented the seeds from sprouting when they are still attached to the mother plant as groundnut pods developed underground. In some crop species, dormancy can be gradually reduced throughout the ensuing after-ripening phase [12, 13]. According to Finch-savage and Bassel [14], germination can occur early in the development of the seed, although desiccation resistance, genomic repair mechanisms and overall seed quality such as capacity to emerge, and vigour can only be developed later.

Biochemical changes in dormant and non-dormant varieties

In dormant variety CO6, the total phenol content of seed gradually increased from 41.50 µg/g at 80 DAS to 90.90 µg/g at 140 DAS. In VRI 8, the phenol content of seed was 33.67 µg/g at 70 DAS and it increased considerably to 52.34 µg/g at 120 DAS (Fig.1) but the concentration was lesser than the variety CO6. In variety CO 6, the total phenol content of the pod gradually increased from 59.87 µg/g at 65 DAS to 98.73 µg/g at 140 DAS. In non dormant variety VRI 8, the quantity of phenol content of pod was lesser than the dormant varietyCO 6 (62.69 µg/g at 120 DAS) (Fig.4).

From the beginning of pod development until maturity, the phenol content in pods of both dormant and non-dormant groundnut varieties increased and later-harvested pods had more phenol deposition. In comparison to non-dormant varieties, the dormant variety had higher phenolic content during maturation. Similar result was obtained by Sreeramulu and Rao [15] Total phenol concentration increased with seed development, and it was greater in dormant seeds at all stages of development. In all phases of development, the levels of phenolic acids were greater in dormant seeds. Similar findings was reported in beet seed [16] and dormant varieties of groundnut [17].

Seed dormancy and germination are regulated by two opposing hormones i.e. ABA which induces dormancy and GA which stimulates seed germination [18]. In the present study, assessment of hormones at maturity stage revealed the presence of high level of ABA (0.912 µg/g) and low level of GA_3 (1.117 µg/g) in dormant CO 6 variety. In contrast, low level of ABA (0.043 µg/g) and high level of GA (1.141 µg/g) was found in non dormant VRI 8 variety (Table 1). Phytohormones present in the seed plays an important role in imposing as well as releasing dormancy. The participation of ABA in the induction and maintenance of dormancy has been extensively studied in different plant species [19]. Maximum level of ABA is accumulated during maturation drying which regulates dormancy induction and maintenance [20]. The dormancy imposition and maintenance depend on an intrinsic balance between the synthesis and catabolism of GA and ABA, which will determine the dominance of either hormone and its downstream signaling cascades [21].

ABA is a positive regulator of dormancy induction and a negative regulator of germination, while GA counteracts

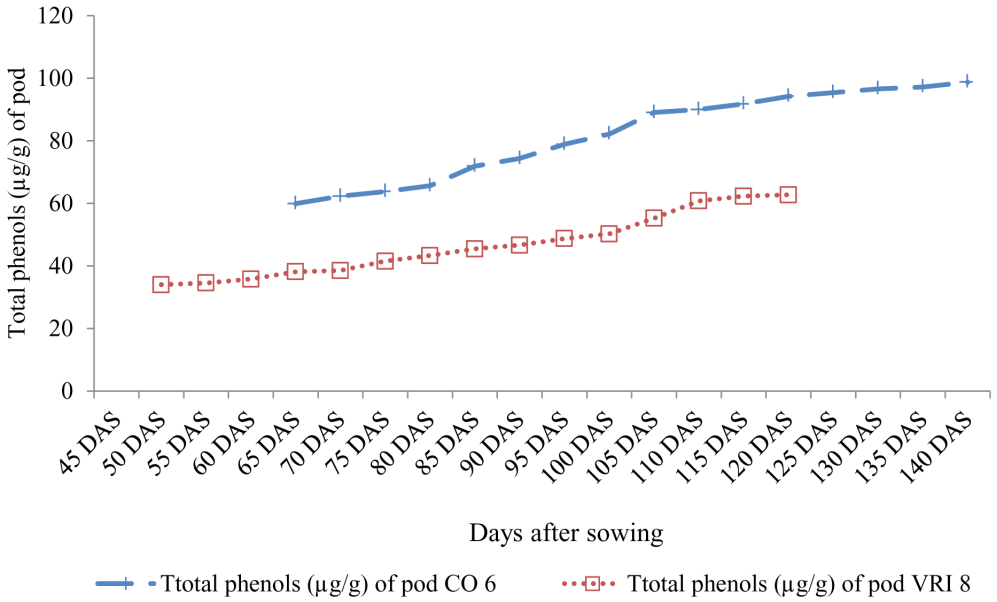

Figure 4. Effect of seed developmental stages on total phenols (µg/g) of pod in groundnut varieties CO 6 and VRI 8

Table 1. Level of hormones in dormant (CO 6) and non-dormant (VRI 8) groundnut varieties at maturity stage

Hormones	Varieties	
	CO 6	VRI 8
GA_3 (µg/g)	1.117	1.141
ABA (µg/g)	0.912	0.043

ABA to release dormancy and promote germination. Similar results of high ABA and low GA content in dormant seeds was reported by Shobha and Renugadevi [22] in groundnut. Dormant seeds maintained a high ABA/GA ratio in groundnut while dormancy release showed increased GA biosynthesis and decreased ABA content [23]. In dormant groundnut seeds, the growth promoters were high during the early growth stage (20-50 DAA) and decreased afterwards while germination inhibitors such as ABA and phenols accumulated; whereas in non-dormant groundnut seeds, the inhibitors increased up to some point of the growth stage (40 DAA) and then declined to favour germination [24]. A study in rice seeds revealed that accumulation of ABA leads to induction of dormancy at the early stage of seed growth and dormant rice varieties showed the presence of ABA and absence of GA_3 in contrast non dormant varieties registered the presence of GA3 and absence of ABA [25]. Thus, dormancy developed gradually during seed maturation resulting in a drastic drop in germination percentage at 135 DAS in CO 6 variety.

The GC-MS is the most preferred technique for qualitative and quantitative analysis of volatile and semi volatile bioactive compounds and used in the present study. In groundnut CO 6, the volatile compounds of different groups were found such as Phenethylamine, 3-Pyridinecarboxylic acid, 9-Octadecenoic acid (Z)-, Decanoic acid, Hexadecanoic acid,3-O-Methyl-d-glucose and Oleic acid (Table 2). In the non-dormant variety VRI 8, 3, 4-dimethyl-, Phenylacetic acid, à-N-Normethadol, Oleic acid, Docosahexaenoic acid and 9-Hexadecenoic acid were detected. The detected compunds were classified based on the functional group and their respective properties were identified (Table 3). It was observed that the compounds responsible for promoting seed germination and also inhibiting seed germination were present in the non dormant and dormant groundnut varieties. In seeds of dormant variety, germination inhibiting compounds such as phenols and deconoic acid were found at maturity stage. Phenols inhibited seed germination and forced the seeds in to dormancy [26]. Decanoic acid is a saturated fatty acid which inhibits the embryo development, structure and function and reduces the growth of coleoptiles [27]. Whereas in seeds of non dormant variety, germination promoting compounds such as phenyl acetic acid and oleic acid were found. Phenyl acetic acid (PAA) is a natural auxin widely distributed in both plants and seeds [28]. PAA plays an important role as an auxin in many aspects of plant growth and development and it promotes and up-regulate the early

Table 2. volatile compounds in CO 6 groundnut variety at 130 days after sowing

S. No.	Compound name	Retention time	Peak area (%)	Group/Class	Properties
1.	Phenethylamine	4.42	9.76	Amine	Insecticidal activity [30]
2.	3-Pyridinecarboxylic acid	0.11	8.98	Carboxylic acid	Anti-inflammatory [31]
3.	9-Octadecenoic acid (Z)	22.07	0.17	Fatty acid	Anti-fungal Anti-bacterial activity [32]
4.	Decanoic acid	23.55	0.10	Fatty acid	Methylguanidine inhibitor, arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity [33]
5.	Hexadecanoic acid	23.92	0.06	Fatty acid	Anti-oxidant and Anti-bacterial [34]
6.	3-O-Methyl-dglucose	28.06	25.74	Carbohydrates	Antioxidant, Antiinflammatory, Antimicrobial activity [31]
7.	Oleic acid	30.66	0.92	Fatty acid	Anti-oxidant and antiinflammatory [32]
8.	Phenol	30.71	0.86	Alcohol	Inhibit cell division and germination, and force the seed into dormancy [35]

Table 3. volatile compounds in VRI 8 groundnut variety at 105 days after sowing

S. No.	Compound name	Retention time	Peak Area (%)	Group/Class	Properties
1.	3,4-dimethyl-	2.24	38.02	Alkyl	Anti-fungal [31]
2.	Phenylacetic acid	3.96	0.35	Carboxylic acid	Growth promotor and plant development [28]
3.	à-N-Normethadol	7.76	0.08	Alcohol	Anti-inflammatory [36]
4.	Oleic acid	25.56	0.03	Fatty acid	Anti-oxidant and antiinflammatory [32]
5.	Docosahexaenoic acid	27.82	0.40	Fatty acid	Antimicrbial activity [33]
6.	9-Hexadecenoic acid	28.45	0.10	Fatty acid	Anti-oxidant , Anti-bacterial [34]

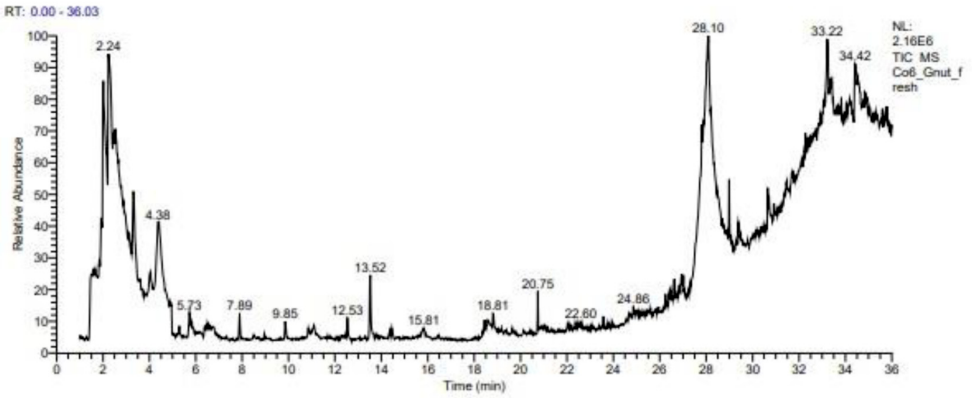

Figure 5. Profile of volatile compounds with peak area and retention time in dormant variety CO 6 at physiological maturity stage (130 Days after sowing)

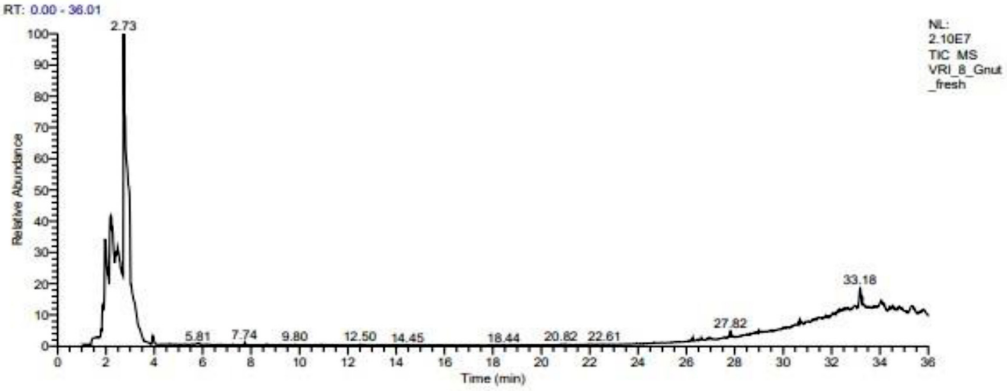

Figure 6. Profile of volatile compounds with peak area and retention time in non-dormant variety VRI 8 at physiological maturity stage (105 Days after sowing)

auxin responsive genes of TIR1/ AFBs with auxin-mediated regulation (Aux/IAAs) [29].

CONCLUSION

The non-dormant variety (VRI 8) recorded maximum seed germination, lower level per cent of fresh ungerminated seeds, phenol content in pods and seeds, low level of ABA and high level of GA and presence of germination promoting compounds such as phenyl acetic acid and oleic acid during seed maturation stage. In contrast, the dormant variety (CO 6) was lowest in germination with high level of fresh ungerminated seed, and found to contain higher phenol content, high level of ABA and low level of GA_3 and presence of germination inhibiting compounds phenols and deconoic acid at maturity stage. This confirms that seed dormancy is present in groundnut CO6 cultivar at physiological maturity stage. The physiological maturity of the dormant seeds could be assessed through physiological indices of pod and physiological characteristics combining with tetrazolium staining pattern of the seeds

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