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Role of Testa, Cotyledons and Embryonic Axis in Seed Dormancy of Groundnut (*Arachis hypogaea* L.)

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With one figure and 4 tables

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Abstract

Experiments were carried out during winter–summer (January–June) season to understand the role of seed testa, cotyledons and embryonic axis in imparting dormancy of some groundnut cultivars belonging to different habit groups. Crop was harvested at four maturity stages; 90, 100, 110 and 121 days after emergence (DAE). After drying the pods in shade for 2 days, the germination of seeds with (GST) and without (GSW) testa in rolled germination towels and seeds, and embryonic axes (GEM) in culture media from individual plants of a cultivar was studied. Seed testa played an important role in imparting dormancy followed by the cotyledons, and embryonic axis. However, the nature of dormancy of embryonic axis appeared to be different from that of the testa and cotyledons. Results suggested that the dormancy in groundnut is regulated mainly by testa (a maternal tissue) in the Spanish type, but by cotyledons, and embryonic axis (both zygotic tissue) as well as testa in Virginia types. Thus the genetic control of seed dormancy in groundnut appears to be quantitative in nature.

Key words: *Arachis hypogaea* L. — groundnut — maturity stage — seed dormancy — testa — cotyledon — embryonic axis

Introduction

In groundnut (*Arachis hypogaea* L.) seed dormancy is predominant in the subspecies *hypogaea* (Virginia type) though dormancy to a certain level may sometimes be found in the varieties *vulgaris* (Spanish type) and *fastigiata* (Valencia type) of the ssp. *fastigiata* (Bailey and Bear 1973). Groundnut seed dormancy, whether a desirable or undesirable characteristic, depends on agronomical practices. In India groundnut is cultivated in the winter–summer period, rainy and post-rainy seasons and prolonged seed dormancy is an undesirable character; however, a short period (10–15 days) dormancy is required in the Spanish type of groundnut to prevent *in situ* seed germination in the field due to unseasonal rains at the time of crop maturity. Therefore, basic information is required to make a successful breeding programme for induction of short period dormancy in Spanish groundnut. Information on the extent and location of dormancy factor(s) in the testa and embryo is contradictory (Stokes and Hall, 1930, Toole et al. 1964, Vaithialingam and Rao 1973a). Patil (1967) concluded that seed dormancy was not associated with embryo but Vaithialingam and Rao (1973b) found that the embryonic extracts of a dormant type induced dormancy in a non-dormant type. The role of the cotyledons in dormancy has not been clearly established. The present investigations attempted to understand the role of testa, cotyledons and embryonic axis in dormancy of the different botanical types of groundnut.

Materials and Methods

Groundnut cultivars belonging to different habit groups (Table 1) were sown in 5 m × 3 m plots at 45 cm × 15 cm spacing in the winter–summer (January–June) season of 1992. The crop was maintained following recommended agronomic practices (ICAR 1987). Ten plants of each cultivar were harvested at 90 (stage 1), 100 (stage 2), 110 (stage 3), and 121 (stage 4) days after emergence and the pods were stripped after 2 days of shade drying. The number of seeds obtained from individual plants varied highly (15–55) from plant to plant and cultivar to cultivar. The testa of half of the seeds from each plant were removed. For germination study the seeds with testa and without testa were placed in wet rolled towels (between paper substrates). The substrates were wrapped in plastic bags and placed in an upright position in test-tube baskets. The baskets were shifted to a germinator at 28 ± 2°C temperature and at 90–95 % relative humidity. After 6 days of incubation, germination percentage of seeds was computed, based on the normal seedlings following ISTA rules (ISTA 1985).

Table 1: Germination percentages of groundnut seeds with and without testa and proportionate increase in germination percentage due to removal of testa at four stages of maturity

Cultivar	Botanical type	Stage 1			Stage 2			Stage 3			Stage 4		
		GST	GSW	PI	GST	GSW	PI	GST	GSW	PI	GST	GSW	PI
ICGV 86590	SB	15	75	4	23	87	3	18	87	4	94	98	0
ICGS (FDRS) 10	SB	53	100	1	35	86	1	47	98	1	95	100	0
IGS 11	SB	3	35	13	4	39	8	1	23	47	16	95	5
ICGS 44	SB	2	5	2	2	17	6	5	47	8	35	54	1
ICGS 5	VB	4	45	11	0	16	72	2	48	21	20	74	3
ICGS 37	VB	2	36	14	9	23	1	10	57	15	22	69	2
ICGS 21	VB	14	28	1	4	25	5	11	58	4	44	95	1
Kadiri 3	VB	2	78	34	1	40	31	7	68	10	16	100	5
5S	VB	2	45	25	2	43	20	8	75	9	14	99	6
NFP 140	VB	2	38	19	1	67	93	12	83	6	16	93	5
RB 90	VB	5	3	0	6	19	2	12	58	4	22	97	3
M 13	VR	3	0	—	0	1	—	0	3	—	2	66	38
GAUG 10	VR	0	0	—	3	10	2	0	1	—	6	51	4
Mean		8	38	11	7	36	22	10	54	1	31	84	3
CV		175	85	100	138	76	142	83	55	1	97	22	79

CV = Percentage coefficient of variation; SB = Spanish bunch (ssp. *fastigiata*); VB = Virginia bunch; VR = Virginia runner (ssp. *hypogaea*); GST = germination of seeds with testa; GSW = germination of seeds without testa; PI = proportionate increase in germination due to removal of testa. The values are means calcuated from GST and GSW of individual plant.

In the case of the seeds obtained from the plants of second, third, and fourth stages of maturity, the germinated seeds were removed after counting and ungerminated seeds were shifted into new rolled towel and were soaked along with the towel in 0.25 % ethrel (2-chloro ethyl phosponic acid). After 5 min soaking the rolled towels were moved to the germinator and incubated at the same temperature as mentioned earlier. The germination percentage of seeds with (EST) and without testa (ESW) was computed, based on the seeds producing radicles of >1 cm length after 4 days of incubation. For each cultivar and stage the proportionate increase in germination due to the removal of testa (PI) was computed as:

$$PI = (GSW - GST)/GST.$$

The average of 10 plants was taken as the germination percentage and PI of a cultivar at a particular stage.

The germination of seeds with and without testa and that of the excised embryonic axis (GEM) *in vitro* was studied at the third and fourth stages of maturity (Table 3). A random sample of 90 seeds was taken from a sample of 5 plants of each cultivar. The seeds were thoroughly washed with distilled water, Tween 80, 70 % ethanol and 0.05 % mercuric chloride. Ten seeds with intact testa, 10 seeds without testa and 10 asceptically-excised embryonic axes were placed on a sterile, hormone-free, MS culture medium (Murashige and Skoog 1962) containing the vitamins of B5 medium (Gamborg et al. 1968) in 7 % agar. Three such replicates constituted the experiment. The petri dishes were incubated at 26 ± 1 °C and 18 h light for 10 days. Germination percentage of the seeds with and without testa was calculated based on the normal seedlings following the ISTA rules (ISTA 1985). The excised embryonic axes which produced normal radicles of >5 mm length were considered germinated.

Results

The germination percentage of seeds with and without testa increased substantially after the third stage in all but M 13 and GAUG 10 (Table 1). The Spanish cultivars had the highest germination percentage at all the stages, though they showed some degree of dormancy until the third stage. There was a wide variation among the Virginia types, and in M 13 and GAUG 10 the germination with testa was much lower even at the fourth stage (Table 1). The removal of the testa in general enhanced germination except in M 13 at the first stage. However, at the fourth stage germination of seeds without testa was higher even in M 13 and GAUG 10. The proportionate increase due to removal of testa varied with the cultivar and stage (Table 1). Except in M 13 and GAUG 10, the ethrel-treated seeds with testa

Table 2: Germination percentages of ethrel-treated seeds with or without testa at three stages of maturity

	Stage 2		Stage 3		Stage 4	
Cultivar	EST	ESW	EST	ESW	EST	ESW
ICGV 86590	100	100	100	100	100	100
ICGS (FDRS) 10	95	100	100	100	100	100
ICGS 11	91	95	99	100	93	100
ICGS 44	88	98	100	100	95	100
ICGS 5	78	100	100	100	85	100
ICGS 37	95	100	99	100	83	100
ICGS 21	91	100	99	100	91	100
Kadiri 3	99	100	100	100	99	100
5S	98	100	100	100	93	100
NFP 140	95	100	98	100	90	100
RB 90	94	100	97	100	89	100
M 13	43	100	42	100	55	100
GAUG 10	84	100	79	100	68	100
Mean	89	99	93	100	88	100
CV	16	2	18	0	15	0

CV = percentage coefficient of variation; EST = germination of seeds with testa after ethrel treatment; ESW = germination of seeds without testa after ethrel treatment.

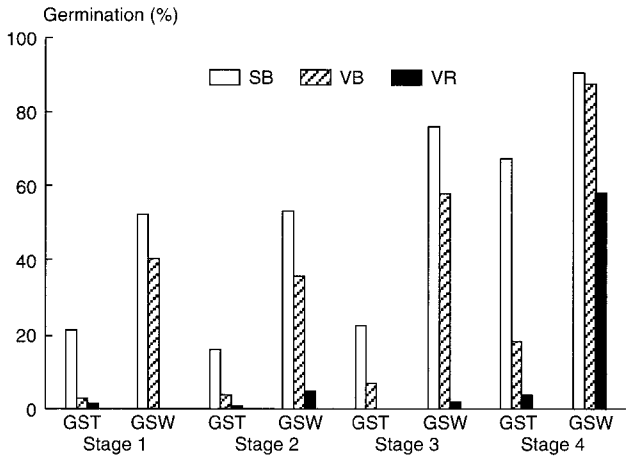

Fig. 1: Germination percentage of seeds with or without testa of different habit groups of groundnut at four stages of maturity. SB = Spanish bunch; VB = Virginia bunch; VR = Virginia runner

showed an almost complete break in dormancy at all the stages (Table 2). The results showed that the removal of testa enhanced germination at all stages in the Spanish type, but at the fourth stage in the Virginia bunch and Virginia runner types (Fig. 1).

In general, the seeds without testa either did not germinate or had low *in vitro* germination. The *in*

Table 3: Germination percentages of seeds with or without testa and embryonic axes in embryo culture medium

	Stage 3			Stage 4		
Cultivar	GST	GSW	GEM	GST	GSW	GEM
ICGS (FDRS) 10	50	100	100	14	—	100
ICGS 11	—	—	80	44	55	100
ICGS 44	—	20	90	—	33	100
5S	—	—	20	15	10	88
RB 90	—	—	60	—	22	100
Kadiri 3	—	—	70	—	10	100
NFP 140	—	—	70	Contaminated		
ICGS 21	20	20	100	11	—	100
M 13	—	—	30	—	—	43
GAUG 10	—	—	40	—	—	60

— = indicates no germination; GST = germination of seeds with testa; GSW = germination of seeds without testa; GEM = germination of excised embryonic axis.

Table 4: Coefficients of correlation between stages of maturity, in all possible pairs, for the variable germination percentages of seed with and without testa

	1 and 2	1 and 3	1 and 4	2 and 3	2 and 4	3 and 4
GST	0.90	0.94	0.81	0.90	0.90	0.82
GSW	0.82	0.75	0.64	0.81	0.67	0.69

P = (0.05); df = 11; GST = germination of seeds with testa; GSW = germination of seeds without testa.

vitro germination of the embryonic axes at the third stage, in general, was high (>60 %) in all except 5S, M 13 and GAUG 10. At the fourth stage the germination percentage was <60 % in M 13 and GAUG 10 (Table 3) and the rest of the genotypes had 100 percent germination.

The coefficient of correlation (r) between germination of seeds with testa and that of seeds without testa was significant at all stages but the fourth. Though the coefficient of variation for germination of seeds with testa was higher than that of seeds without testa at all the stages, the magnitude was higher at the fourth stage (Table 1). The coefficient of correlation between germination of ethrel-treated seeds with testa and germination of untreated seeds with testa was significant at the fourth stage only ($r = 0.58$). The germination percentage of ethrel-treated seeds with testa and germination percentage of seeds without testa was significantly correlated at all the three stages ($r = 0.60, 0.68, 0.64$ at the second,

third and fourth stages respectively). Coefficients of correlation among the stages in all possible pairs were significant for both GST and GSW (Table 4).

Discussion

The break in dormancy by ethrel, irrespective of the cultivar and maturity status of the seeds, indicated that the lower germination at early stages of maturity was primarily due to an inhibitory mechanism rather than the lack of physiological maturity of the seed. The comparatively lesser effect of ethrel on seeds with and without testa in the cv. M 13 reflected the control of dormancy both in the testa and cotyledons. The increase in germination caused by the removal of testa at all the stages indicated the predominant role of testa in dormancy. The role of cotyledons and/or the embryonic axis in dormancy was evident from the facts that the germination percentage of seeds without testa was quite low (0–58 %) in the Virginia runner types and even in the Spanish types, this was 52–90 % i.e. below 100 % and only ethrel treatment could cause full germination in seeds with or without testa. The proportionate increase in germination due to removal of testa (PI) therefore, was a comparative measure of the role of testa when compared with the rest of the seed. A good degree of genotypic variation in PI was observed. However, it was difficult to quantify the predominance of the role because of the lack of any specific trend in the PI values and correlation between stages for PI. The lesser proportionate increase in germination due to removal of testa at the fourth stage of maturity was because the role of the cotyledons and/or the embryonic axis in dormancy diminished when the seeds matured beyond a certain stage. The lack of significant correlation between germination of seeds with and without testa at the fourth stage further corroborated this.

The germination percentage of the embryonic axis at stage three and four were much higher than or comparable to the germination of seeds without testa at the corresponding stages, barring a few cases (Tables 1 and 3). The values for M 13 and GAUG 10 were especially notable. In stage four, germination of embryonic axes of all cultivars, except for the Virginia runner types, were almost 100 % whereas this was not so for germination of seeds without testa. Therefore, if the embryonic axis had a role in dormancy it would probably be lesser than that of the cotyledons. The absence of any significant correlation at either stage three or stage four between germination percentage of seeds with testa and the embryonic axes and that between germination percentage of seeds without testa and embryonic axes indicated a possible difference in the bases of the behaviours of the embryonic axes on the one hand, and the testa and the cotyledons on the other, with respect to dormancy.

Some cultivars (ICGS 11, ICGS 44, and ICGS 37) used in the experiment are selections from the cultivar Robut 33-1 and some (ICGS 5, 5S, RB 90) had the same cultivar in their pedigree. However, there was no perceptible commonalty in their dormancy behaviour. This might be due to the quantitative nature of inheritance of seed dormancy in groundnut as proposed by Hull (1937). It appeared that dormancy is regulated mainly by seed testa (a maternal tissue) in both the Spanish and Virginia types, but in the Virginia types the cotyledons and embryonic axis (both zygotic tissues) also had important roles. The dormancy factor(s) in the genotypes with prolonged seed dormancy is located in seed coat, cotyledon and embryonic axis, whereas for the genotypes with short dormancy period the factor(s) is located in the seed coat only.

Conclusions

Small and unequal sample size, and various degree of maturity of the seeds from the same plant are the inevitable handicaps in this study. Despite this handicap the general trends of the study could bring out certain aspects of dormancy in groundnut fairly clearly. Testa, the maternal tissue, the cotyledons and possibly the embryonic axis (the zygotic tissue) had definite roles in controlling dormancy. Though the role of testa appeared to be greater than the others, the relative predominance of these components in individual varieties could not be judged in this study. Experiments with more varied genetic material and detailed *in vitro* studies are required to explain the nature of inheritance of seed dormancy in groundnut.

Zusammenfassung

Bedeutung der Testa, Kotyledonen und Embryonenaxe in der Samenruhe von Erdnuß (*Arachis hypogaea* L.)

Ws wurden Experiment durchgeführt während der Winter–Sommersaison (Januar–Juni), um die Bedeutung der Samentesta, der Kotyledonen und der Embryonenaxe hinsichtlich ihrer Auswirkung auf die Dormanz einiger Erdnußkultivare unterschiedlicher Wachstumsgruppen zu untersuchen. Der Bestand wurde zu vier unterschiedlichen Reifestadien 90, 100, 110 und 121 Tage nach

Auflaufen (DAE) geerntet. Nach dem Trocknen der Hülsen im Schatten für zwei Tage wurde die Keimung der Samen mit (GSD) und ohne (GSW) Testa, Samen in vitro, und Embryonenaxen (GEM) in Kulturmedian, von einzelnen Pflanzen der Kultivare untersucht. Die Samentesta spielte eine bedeutende Rolle in der Entwicklung der Dormanz, gefolgt von den Kotyledonen und den Embryonenaxen. Allerdings, die Natur des Ruhezustandes der Embryonenaxen schien sich von der der Testa und Kotyledonen zu unterscheiden. Die Ergebnisse weisen daraufhin, daß die Dormanz bei Erdnuß im wesentlichen durch die Testa (ein mütterliches Gewebe) bei den spanischen Buschtypen und durch Kotyledonen und Embryonenaxen (beides zygotische Gewebe) neben der Testa in den Virginiatypen reguliert wird. Die genetische Kontrolle der Samenruhe bei Erdnuß erscheint daher quantitativer Natur zu sein.

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