Imbibition rates, leachates and fungal colonization of seeds of selected groundnut germplasm lines with different seed test colours(1)

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Abstract. — Relationships between seed testa colour, rate of water uptake, seed damage, seed leachates, and *in vitro* seed colonization and *in vivo* seed rots/aflaroot diseases by *Aspergillus flavus* were examined in various groundnut germplasm lines belonging to different botanical types with different seed coat colours. No relationship between seed coat colour and water uptake rates was evident. Slow water uptake appeared to be related to increased seed damage, though it may be more influenced by other characteristics such as seed size. White-seeded lines were highly susceptible to *in vitro* seed colonization by *A. flavus*, but no correlation existed between seed coat colour and seed rots and aflaroot diseases caused by the fungus.

Key words. — *Arachis hypogaea, Aspergillus flavus*, imbibition rates, leachates, seed colonization, aflaroot.

INTRODUCTION

Poor plant stand in groundnut fields is of common occurence, particularly in the semi-arid tropics. This has generally been attributed to low vigour and low viability of seed, and seed and seedling disease problems arising from mainly seed-borne infections by fungi such as *Aspergillus flavus* Link ex Fr. and *A. niger* Van Tieghem. Seed dressing fungicides have been shown to provide limited protection against such pathogens (Gibson and Clinton, 1953; Young *et al.*, 1969). Several crop seeds with rapid imbibition rates exhibit low vigour and poor emergence, and are more prone to fungal infection than seeds with slower water uptake rates (Powell, 1989). In groundnuts, wax deposits on seed coat contributed to low permeability leading to resistance to seed invasion and colonization by *A. flavus* (Ketring *et al.*, 1976, La Prade *et al.*, 1973). Groundnut genotypes with coloured testa exhibited greater resistance to seed invasion by *A. flavus* than genotypes with white or variegated testa (Carter, 1970, 1973). Tanin-like compounds and polypeptides in seed coat may confer resistance to seed invasion by fungi (Pettit *et al.*, 1989).

In the present study influence of seed testa colour, rate of water uptake, seed damage, seed leachates on seed invasion/colonization and seed rot/aflaroot diseases caused by *A. flavus* in selected groundnut germplasm lines belonging to different botanical types were investigated.

MATERIALS AND METHODS

❑ Genotypes

Sixteen genotypes (four each from the four botanical types, viz. Spanish, Valencia, Virginia Bunch (VB), and Virginia Runner (VR) with different seed testa colours were used (Table I). Seed of the genotypes were obtained from the 1989 rainy season crops grown at the ICRISAT Center farm. Experiments were conducted in completely randomised designs with 3 replications.

❑ Imbibition

The rate of water uptake was determined on 10 g seed samples held between filter papers (Whatman, 9 cm diameter) moistened with 10 ml of sterile, distilled water. Imbibition rates after 1, 3, 4, 8, 24, and 32 h were determined by measuring increase in seed weight following imbibition and expressed in mg per gram of seed in unit time (hours) (Ketring *et al.* 1976).

The extent of tissue damage on the cotyledon surface after imbibition was also assessed. This was done by removing testae from imbibed seeds and immersing them in a 1.0% solution of tetrazolium salt at 30°C for 16 h. The extent of non-stainibility, which is indicative of damaged tissue was assessed on abaxial surface of cotyledons (Powell and Mathews 1978).

❑ Seed leachates

Seed leachates were collected by immersing 10 g seeds in 75 ml of sterile deionized water for 8, 24, 32, 48 and 72 hrs. Electrical conductivity (EC) of the leachates was measured using an electrical conductivity meter (YSR Model 32). The EC reflected the amount of electrolytes (saltes) in the leachates. Leachates were analysed for polyphenol contents using the prussian blue method (Price and Butler, 1977).

❑ *A. flavus* infection/colonization

Twenty seed of each genotype were inoculated (by smearing the seeds with inoculum in petri plates) with a toxigenic strain of *A. flavus* (AF 8-3-2A) and sown in plastic pots (15 cm diameter) containing a mixture of sand: soil: manure (1:2:1). Three replications were used. Inoculum of the fungus was also mixed with the upper 10 cm layer of top soil. Seed rot due to *A. flavus* and aflaroot disease incidences were recorded between 8-28 days after sowing.

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TABLE I. — **Rate of water uptake, seed leachates conductivity, polyphenols and *in vitro* seed colonization and seed/seedling rots by *A. flavus* in groundnut lines with different seed coat colors**

ICG No	Seed coat colour	Water uptake[1] (in mg) 1 h	Tissue damage[2] (24 h)	Leachates[3] conductivity	Leachates[4] polyphenols	Seed[5] colonization (%)	Seed rot[6] aflaroot disease
A. hypogaea ssp *fastigiata* var. *fastigiata* (Valencia)							
7497	White	0.80 ± 0.17	16.0 ± 10.82	0.12 ± 0.00	0.03 ± 0.00	88	16.7 ± 3.30
10086	Tan	1.42 ± 0.01	10.8 ± 5.15	0.24 ± 0.00	0.55 ± 0.01	43	18.3 ± 1.70
2738	Red	0.96 ± 0.19	3.8 ± 1.75	0.13 ± 0.00	0.46 ± 0.01	24	13.3 ± 1.70
6571	Purple	0.99 ± 0.12	5.00 ± 1.78	0.15 ± 0.00	0.40 ± 0.01	22	13.3 ± 4.4
A. hypogaea ssp *fastigiata* var. *vulgaris* (Spanish)							
432	White	0.94 ± 0.07	17.0 ± 12.82	0.29 ±0.01	0.04 ± 0.00	88	70.0 ± 5.8
2960	Tan	0.89 ± 0.07	7.3 ± 2.56	0.26 ± 0.01	0.53 ± 0.01	23	30.0 ± 0.0
8358	Red	0.76 ± 0.14	7.5 ± 3.79	0.14 ± 0.00	0.40 ± 0.00	25	18.3 ± 1.7
8850	Purple	0.76 ± 0.12	1.3 ± 0.47	0.12 ± 0.00	0.57 ± 0.01	15	5.0 ± 2.9
A. hypogaea ssp *hypogaea* var. *hypogaea* (Bunch)							
7789	White	1.10 ± 0.01	4.8 ± 1.03	0.40 ± 0.07	0.38 ± 0.14	89	5.0 ± 2.9
174	Tan	0.63 ± 0.13	24.8 ± 4.32	0.44 ± 0.01	0.75 ± 0.00	32	68.3 ± 7.3
2481	Red	0.80 ±0.14	9.3 ± 3.17	0.16 ± 0.00	0.79 ± 0.01	20	8.3 ± 1.7
7431	Purple	1.00 ± 0.05	5.8 ± 1.79	0.26 ± 0.00	0.52 0.01	18	6.6 ±1.7
A. hypogaea ssp *hypogaea* var. *hypogaea* (Runner)							
3790	White	1.11 ± 0.03	4.8 ± 1.31	0.26 ± 0.00	0.07 ± 0.00	90	25.0 ± 7.6
4923	Tan	0.73 ± 0.09	4.0 ± 1.35	0.15 ± 0.01	1.02 ± 0.06	33	23.3 ± 8.8
6109	Red	0.83 ± 0.11	5.5 ± 2.39	0.25 ± 0.01	0.26 ± 0.00	9	20.0 ± 5.0
5040	Purple	1.23 ± 0.00	10.5 ± 4.59	0.58 ± 0.00	0.95 ± 0.00	49	0.0 ± 0.0

(1) Imbibition rate
(2) Tissue damage to cotyledon surface of seed on soaking in water
(3) Electrical conductivity of leachates (salts)
(4) Polyphenols concentration in leachates
(5) *In vitro* seed colonization by *Aspergillus flavus*
(6) Seed rots and aflaroot disease caused by *A. flavus*

The genotypes were also evaluated for *in vitro* seed colonization by *A. flavus* (Mehan and McDonald, 1980).

❑ Statistical analysis

Analyses of variance were performed on the data, using the genotypes as groups on the basis of botanical types and seed coat colour. Significance tests were carried out between groups and within groups. Correlation coefficients were calculated for quantitative traits, percent seed colonization and seed rot and aflaroot disease.

RESULTS

❑ Imbibition

• Rate of water uptake

Water uptake was similar in all genotypes. Maximum water uptake occured in the first hour followed by a sharp decrease by the third hour. It reached a plateau between 3rd and 8th hour and fell in subsequent hours. In the first hour, lines differed significantly for imbibition rates between and within the botanical groups except for Spanish types (Table I). Valencia types with white seed testa had significantly lower imbibition rates than lines with other testa colours (Table I). Virginia types with tan and red testa showed significantly lower imbibition rates than those white and purple testa. Thus there were no significant differences between red and white testa group and significant differences (P. 05) were noted within tan and purple testa groups (Table I).

• Seed damage

In general, seed damage was more in lines with white testa. It ranged from 1.3 to 24.8% of total abaxial surface of cotyledons, the highest being in the genotype (ICG 174) with large seed size. No correlation was found between seed damage and seed coat colour. In Spanish and Valencia types, lines with white testa showed significantly higher damage than lines with coloured testa.

❑ Leachates

• Electrical conductivity

No clear cut patterns in the electrical conductivity of leachates were observed (Table I). The pH of these leachates was similar among various botanical types.

• Polyphenol concentrations

In general, the leachates of seeds with white testa had low concentrations of polyphenols (Table I). The Valencia and Spanish lines had the lowest concentrations (0.048 and 0.034, respectively) followed by the lines in the VR and VB groups. Tan testa seeds had higher polyphenols (0.53-1.02) than other coloured seeds testa in all botanical groups. Nevertheless, the cultivars within different botanical types and with same seed coat colour differed significantly for polyphenol content.

❑ Susceptibility to *Aspergillus flavus*

• *In vitro* seed colonization

White testa lines showed high susceptibility to seed colonization irrespective of botanical types. Spanish and Valencia lines with red and purple seed testa showed significantly lower percentages of seed colonized by *A. flavus*. The same was also true for the lines in the Virginia Bunch types.

• Seed rot and aflaroot disease

Most of the lines with white seed testa showed high (70% disease incidence) to moderate susceptibility (15-25% disease incidence) to seed rot and aflaroot disease caused by *A. flavus*, except for the white-seeded (ICG 7789) cultivar belonging to VB type which showed low susceptibility (5-15% disease incidence). No significant differences were observed between the cultivars with other seed coat colours. However, the lines within a botanical type differed significantly in their susceptibility to *A. flavus*.

No significant correlations were observed among water uptake, seed damage, electrical conductivity or concentration of polyphenol, and percent seed colonization and seed and seedling disease (Table I).

DISCUSSION

Seed coat colour was not associated with imbibition rate in the range of groundnut genotypes tested. Studies in other crop species have indicated that pigmentation of seed coat was associated with reduced rates of imbibition, vigour and emergence (Powell, 1989). In present study a lot of variability for imbibition rate was evident among the genotypes belonging to different botanical group irrespective of their seed coat color. Thus it is possible to develop/select genotypes with desirable seed coat colour and imbibition rates in different botanical types.

Slow water uptake appeared to be related to increased seed damage leading to high incidence of seed rots and aflaroot disease, contrary to observations by Ketring *et al.* (1976). Our findings suggest that slow and prolonged water uptake can cause more damage to the cotyledons, resulting in reduction in germination, increased infection by soil fungi and poor seedling health. This can also be associated with some other seed characteristics such as seed size, as maximum cotyledon damage was observed in ICG 174 that had largest seed size (100-seed wt. = 66 g).

White-seeded lines were shown to be highly susceptible to *in vitro* seed colonization by *A. flavus*. No such correlation was observed between seed testa colour and susceptibility to seed rots and aflaroot disease, although a few lines with white testa showed high to moderate incidences of these diseases. These results are in disagreement with the findings of Carter (1970, 1973) who observed a strong correlation between testa colour and susceptibility to *A. flavus* seed rots and seedling diseases: coloured-seed cultivars being resistant. These results were further supported while running such correlation analysis for seed colonization by *A. flavus* in 64 randomly selected genotyes with different colours. In the present investigations, some white-seeded lines (e.g. ICG 7789) showed low susceptibility to *A. flavus* seed rots and aflaroot. High susceptibility of the white-seeded lines to *A. flavus* colonization may be attributed to low levels of polyphenols (Jambunathan *et al.*, 1989) However, all white testa lines should not be assumed to show similar susceptibility to invasion by *A. flavus* and other soil fungi under field conditions, because of their differential pod and seed characters, as observed with ICG 7497. This line showed low susceptibility to seed rot and aflaroot disease inspite of low polyphenols; compact thin testa adhering to cotyledons appeared to restrict fungal invasion. Polyphenol contents did not appear to be correlated with resistance in coloured testa lines.

The results highlight the existence of wide variability in seed coat characteristics in lines belonging to different botanical groups. This is also true for the variability within the different seed coat colour groups. Therefore, for improvement of seed coat characteristics, selection and hybridization of genotypes within different botanical groups can achieve desired results.

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RESUME

Taux d'imbibition, éléments lessivés et colonisation fongique chez des graines de lignées sélectionnées d'arachide présentant un tegument de couleurs différentes

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Les rapports entre la couleur du tégument, la vitesse d'absorption d'eau, les dégâts subis par les graines, les éléments lessivés des graines, la colonisation des graines *in vitro* ainsi que les pourritures des graines et la maladie "aflaroot" *in vivo* dues à l'*Aspergillus flavus* ont été étudiés sur diverses lignées d'arachide appartenant à des types botaniques différents présentant un tégument de couleurs différentes. Aucun rapport n'a été observé entre la couleur du tégument et la vitesse d'absorption d'eau. Une absorption d'eau lente semble être liée à des dégâts plus importants au niveau des graines, mais d'autres caractéristiques pourraient y jouer un rôle plus important, notamment la taille des graines. Les lignées à graines blanches sont très sensibles à la colonisation *in vitro* des graines par *A. flavus*, mais il n'existe pas de corrélation entre la couleur du tégument et la pourriture des graines et la maladie "aflaroot" provoquées par le champignon.

Mots clés. — *Arachis hypogaea*, *Aspergillus flavus*, vitesse d'imbibition, éléments lessivés, colonisation des graines, "aflaroot"

RESUMEN

Porcentaje de imbibición, elementos lixiviados y colonización por los hongos en semillas de líneas seleccionadas de maní con tegumento de colores distintos

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Las relaciones entre el color del tegumento, la velocidad de absorción del agua, los daños que sufren las semillas, los elementos lixiviados de las semillas, la colonización de las semillas *in vitro* como también las pudriciones de las semillas y la enfermedad llamada "aflaroot" *in vivo* producidas por *Aspergillus flavus* se estudiaron en diversas líneas de maní pertenecientes a tipos botánicos distintos provistos de un tegumento de colores distintos. Ninguna relación ha sido observada entre el color del tegumento y la velocidad de absorción del agua. Una absorción de agua lenta parece relacionada con daños más importantes para las semillas, pero otras características podrían desempeñar un papel más importante, en especial el tamaño de las semillas. Las líneas de semillas blancas son muy sensibles a la colonización *in vitro* de las semillas por *A. flavus*, pero no hay correlación entre el color del tegumento, la pudrición de las semillas y la enfermedad llamada "aflaroot" producidas por el hongo.

Palabras claves. — *Arachis hypogaea, Aspergillus flavus*, velocidad de imbibición, elementos lixiviados, colonización de las semillas, "aflaroot".