

Effects of moisture stress on germination and protein synthesis in root tips of cowpea (*Vigna unguiculata* L. Walp)

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ABSTRACT

Unpigmented cowpea seeds, TVX 3236 (cream and brown) and IT81S-818 (white), were subjected to more stressful constant moisture conditions (-0.1 and -0.15 MPa) for 10 days. Germination was reduced from 90 to 43 and 60 per cent, respectively, compared to 90 per cent in those subjected to readily water availability (-0.0015 Mpa). These seeds germinated at -0.0015 MPa for shorter periods of 12 and 24 h compared to 48 h before imposition of stress (-0.15 MPa) for 7 days and showed more reduced final germination, after they had been returned to readily water availability for a further 7 days. However, pigmented seeds, TVX 2724-01F (brown) and IT82D-32 (dark-brown), showed very high germination between 90 and 100 per cent even under stress. The difference between these two groups of seeds, therefore, lies in their abilities to withstand water stress which was related to the differences in damage caused to their embryonic cells as shown by the extent of staining with 2, 3, 5 triphenyl tetrazolium chloride (TTC), and their abilities to synthesize protein after stress by incorporating L-[4,5-³H] leucine into their root tips.

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RÉSUMÉ

ASIEDU, E. A., POWELL, A. A. & STRUCHBURY, T.: *Effets de la pression d'humidité sur la germination et la synthèse de protéine dans les bouts de racine de dolique (Vigna unguiculata L. Walp)*. Les graines de dolique non pigmentées, TVX 3236 (crème et brune) et IT81S-818 (blanche), étaient exposées aux conditions d'humidité constantes plus stressantes (-0.1 et -0.15 MPa) pour 10 jours; Ces graines ont subi de germination réduite respectivement de 90 à 43 et 60% comparé à 90% en celles qui étaient exposées à l'eau facilement disponible (-0.0015 Mpa). Ces graines germaient à -0.0015 MPa pour des périodes plus courtes de 12 et 24 h comparées à 48 h avant l'imposition de pression (-0.15 MPa) pour 7 jours et montraient de germination final plus réduite après avoir été remises à l'eau facilement disponible pour 7 jours. Les graines pigmentées TVX 2724-01F (brune) et IT82D-32 (brune-foncée), montraient cependant de germination très élevée entre 90 et 100% même sous les conditions de pression. La différence entre ces deux groupes de graines réside donc dans leurs capacités de résister à la pression d'eau qui était liée aux différences en dégâts causés aux cellules embryonnaires comme révélé par l'ampleur de tacher avec 2, 3, 5 chlorure de triphényl tétrazolium (CTT), et leurs capacités de produire synthétiquement la protéine après la pression par l'incorporation de L-[4, 5-³H] leucine dans les bouts de racine.

Introduction

The inability of viable seeds to germinate, or fail to emerge in the field leads to yield reduction and the need for re-seeding (Hunter & Erickson, 1952). When seeds are sown in the field, they are often subjected to drought stress which results in poor field emergence (AOSA, 2001). In Ghana and most African countries, farmers sow their seeds soon after rainfall. However, these seeds may be subjected to water stress if the rains do not

continue. Germination of seeds under such stress may differ among species, cultivar and seed lot. While vigorous seeds may germinate over a wider range of moisture levels, non-vigorous seeds may only germinate and develop into a productive plant when soil moisture is optimum (Asiedu, Struchbury & Powell, 2000; Heydecker, 1977).

Cultivar differences in seed vigour have been observed in several leguminous species including dwarf French bean (Powell, Oliveira & Matthews,

1986a,b), cowpea (Legesse & Powell, 1992; Asiedu & Powell, 1998; Asiedu & Powell, 2000; Asiedu *et al.*, 2000), and long bean (Abdullah, Powell & Matthews, 1992) in which cultivars with unpigmented seed coats showed poor storability, high levels of imbibitional damage to cells, reduced germination, and poor field emergence.

This study aims at determining the differences among cowpea cultivars, differing in seed coat colour, in their response to water stress during germination.

Materials and methods

The cowpea cultivars used in the study originated from the International Institute of Tropical Agriculture and were produced in Ghana. These were IT81S-818 (a white-seeded, black-eyed cultivar known in Ghana as Bengpla), TVX 3236 (a cream and brown-seeded cultivar), TVX 2724-01F (a brown cultivar known in Ghana as Soronko), and IT82D-32 (a dark-brown cultivar known in Ghana as Asontem).

Seeds were germinated between two Whatman No. 42 filter papers (measuring 10.5 cm in diameter) at the matric potentials of -0.0015, -0.1 and -0.15 MPa. The matric potentials of the filter papers were determined using the calibration curve of Fawcett & Collis-George (1967). The filter papers were oven-dried at 105 °C for 1h, wrapped in aluminum foil and kept in desiccators. Each filter paper was divided into 10 equal concentric segments, and one seed was placed in each segment, on a circle drawn halfway (by area) across the segments. The water content of the filter papers corresponding to the matric potentials of -0.0015, -0.1 and -0.15 MPa were 163.4, 44.0 and 40.0 per cent, respectively, of the dry weight. Deionized water was sprayed uniformly on the filter papers for the desired matric potentials before the seeds were placed between them after damaged and discoloured seeds had been removed.

Ten seeds per replicate of four were germinated at constant -0.0015, -0.1 and -0.15 MPa for 10 days. Ten additional seeds per replicate were germinated

at -0.0015 MPa for 12, 24, or 48 h after which they were transferred to a more stressful condition of -0.15 MPa which was maintained until the 7th day of germination. The seeds were then transferred back to -0.0015 MPa for a further 7 days to determine the final germination. The weights of the filter papers were maintained by periodically removing the seeds and adding more deionized water. Damage to embryonic axes and cotyledons caused by moisture stress were assessed by taking four replicates of 10 seeds of each cultivar and exposing them to -0.0015, -0.1, and -0.15 MPa for 24 h. The embryonic axes were then separated from the cotyledons and both stained using 1 per cent (w/v) 2, 3, 5 triphenyl tetrazolium chloride (TTC) for 3 h at 30 °C. The extent of vital staining of the axes and cotyledons were assessed and categorized as 100, 99-50, 49-1, and 0 per cent stained.

Protein synthesis (incorporation of L-[4,5-³H] leucine) in germinating root tips was determined in the white-seeded cultivar, IT81S-818, and the brown-seeded cultivar, TVX 2724-01F, which had earlier been susceptible to and tolerant of moisture stresses, respectively. Three replicates of 20 seeds were germinated between two filter papers in Petri dishes at readily water availability, -0.0015 MPa for 24 h. Protein synthesis in the germinating root tips was then determined before seeds were transferred to a more stressful condition, -0.15 MPa, for a further 48 and 96 h and protein synthesis determined again at the end of both periods. At the end of 96 h, the seeds were transferred back to -0.0015 MPa for a further 72 h and protein synthesis determined once more.

In another study, two sets of seeds of the tolerant cultivar, TVX 2724-01F (brown-seeded), each containing three replicates of 20 seeds, were germinated at -0.0015 MPa for 48 h during which protein synthesis was determined at 24 and 48 h. After 48 h, one set of seeds was transferred to a more stressful condition, -0.15 MPa, for a further 48 h (i.e., until 96 h). The other set was maintained at -0.0015 MPa for the same period after which protein synthesis was determined. All the studies

were carried out at 22 to 24 °C.

Protein synthesis was measured by the pulse-labeling method and by determining total uptake and incorporation of radioactivity into TCA-insoluble material (Dell' Aquila, 1992). After the required times of germination, samples of 20 5-mm root tips were dissected from the seeds, weighed and transferred to the labeling medium (1.0 ml) containing 0.89 ml sterile water, 10 µl Penicillin/Streptomycin solution (SIGMA P0906, containing 5000 U ml⁻¹ Penicillin G and 5 mg ml⁻¹ Streptomycin), and 0.1 ml L-[4,5-³H] leucine (specific activity 3.15 TBq mmol⁻¹; radioactive concentration 0.74 MBq ml⁻¹)(Amersham International Plc, product no. TRK 170).

After pulse-labeling for 1h at 20 °C in the dark, the root tips were collected and washed four times with 1 ml ice-cold 80 per cent ethanol containing 12 mM L-leucine and then incubated in 2 ml of the same medium for 20 min. Root tips were then homogenized in a small teflon and glass homogenizer in 1 ml of this medium and centrifuged at 2000 x g. The resulting pellet was re-suspended in 1 ml 10 per cent TCA (trichloroacetic acid), heated at 90 °C for 5 min, and then cooled in ice. This was then filtered through GF/C Whatman glass filters (3 cm) and washed with 5 ml 5 per cent TCA followed by 5 ml 80 per cent ethanol. The filter was dried over silica gel in a desiccator for 24 h. The dried pellet was suspended in 20 ml scintillation fluid and stored in the dark for a further 24 h, after which it was

counted in a scintillation counter (Packard Model 2000 CA).

Experimental design was completely randomized block and data were analyzed using the MSTAT statistical package.

Results and discussion

Germination under constant stress led to reduced final germination in unpigmented seeds compared to the pigmented ones (Table 1). Even when subjected to readily water availability, the earlier the unpigmented seeds were transferred from -0.0015 Mpa to the more stressful conditions of -0.15 Mpa, the more pronounced the effect of moisture stress in germination (Table 2). The pigmented cultivar showed a greater ability to retain viable cells in the embryonic axes and cotyledons as indicated by high levels of vital staining using TCC (Table 3), and also to synthesize protein by incorporating large quantities of L-[4,5-³H] leucine into their root tips (Fig. 1a.). Protein synthesis in the first set of pigmented seeds transferred to a more stressful condition, -0.15 MPa for 48 h, after 48 h readily water availability remained constantly low (7 DPM/FW × 10⁴) of L-[4, 5-³H] leucine; whereas the second set, which remained unstressed for the same period, showed a dramatic increase from 7 to 45 DPM/FW × 10⁴ (Fig. 1b).

The equal and unchanging levels of L-[4, 5-³H] leucine during the stress period from 24 to 120 h (Fig. 1a) showed that protein synthesis in

TABLE 1

Germination (% Radicle Emergence) After 10 Days at Constant Matric Potential

<i>Matric potential (Mpa)</i>	<i>Cowpea variety</i>			
	<i>TVX 3236 (Cream and brown)</i>	<i>IT81S-818 (White)</i>	<i>TVX 2724-01F (Brown)</i>	<i>IT82D-32 (Dark-brown)</i>
-0.0015	90.0 b	90.0 b	100.0 a	100.0 a
-0.10	55.0 d	90.0 b	100.0 a	100.0 a
-0.15	43.0 e	60.0 d	90.0 b	100.0 a

Means of columns and rows followed by different letters differ significantly ($P < 0.05$) by Duncan's Multiple Range Test

TABLE 2
Germination (% Radicle Emergence) of Seeds Subjected to Moisture Stress at -0.15 MPa After Transfer From -0.0015 MPa

Time of transfer (h)	Cowpea variety			
	TVX 32336 (Cream and brown)	IT81S-818 (White)	TVX 2724-01F (Brown)	IT82D-32 (Dark-brown)
48	90.0 b	80.0 c	100.0 a	100.0 a
24	75.0 cd	75.0 c	100.0 a	95.0 a
12	65.0 e	70.0 c	100.0 a	95.0 a

Means of columns and rows followed by different letters differ significantly ($P < 0.05$) by Duncan's Multiple Range Test

TABLE 3
Axes or Cotyledons With Complete Vital Staining (%) After Seeds Had Imbibed Water at Either -0.0015 MPa or -0.15 MPa for 24 h

Cowpea variety	Matric potential (MPa)			
	-0.0015		-0.15	
	Axes	Cotyledons	Axes	Cotyledons
TVX 3236 (Cream and brown)	90	80	60	78
IT81S-818 (White)	100	100	70	95
TVX 2724-01F (Brown)	100	100	100	100
IT82D-32 (Dark-brown)	90	95	90	95

pigmented and unpigmented seeds was equally retarded under stress. The pigmented cultivar germinated well after stress because of its ability to retain viable embryonic cells as shown by vital staining using TCC (Table 3), and to synthesize large amounts of protein by incorporating L-[4,5- ^3H] leucine into their root tips (Fig. 1a).

The inability of the second set of pigmented seeds to show increases in incorporating L-[4,5- ^3H] leucine into their root tips during the period of reduced water availability underscores the need for uninterrupted supply of water to cowpea during germination and seedling growth, even in drought-tolerant cultivars. These results agree with those of similar studies in which protein synthesis ceased as stress occurred but started

immediately the seeds were hydrated (Bewly & Black, 1994; Dhindsa & Cleland, 1975; Dell'Aquila & Taranto, 1986; Dell'Aquila, 1992; Lalonde & Bewly, 1986; Dell'Aquila & Bewly, 1989). Thus, although germination and root growth (incorporating L-[4,5- ^3H] leucine into root tips) in both seed types were equally retarded under water stress, the pigmented cultivar had a greater ability to retain viable cells and recover at the resumption of readily water availability. Despite its ability to tolerate water stress, the pigmented cultivar stagnated during the period of reduced water availability, underscoring the need for uninterrupted water supply during germination of cowpea, even in stress-tolerant cultivars.

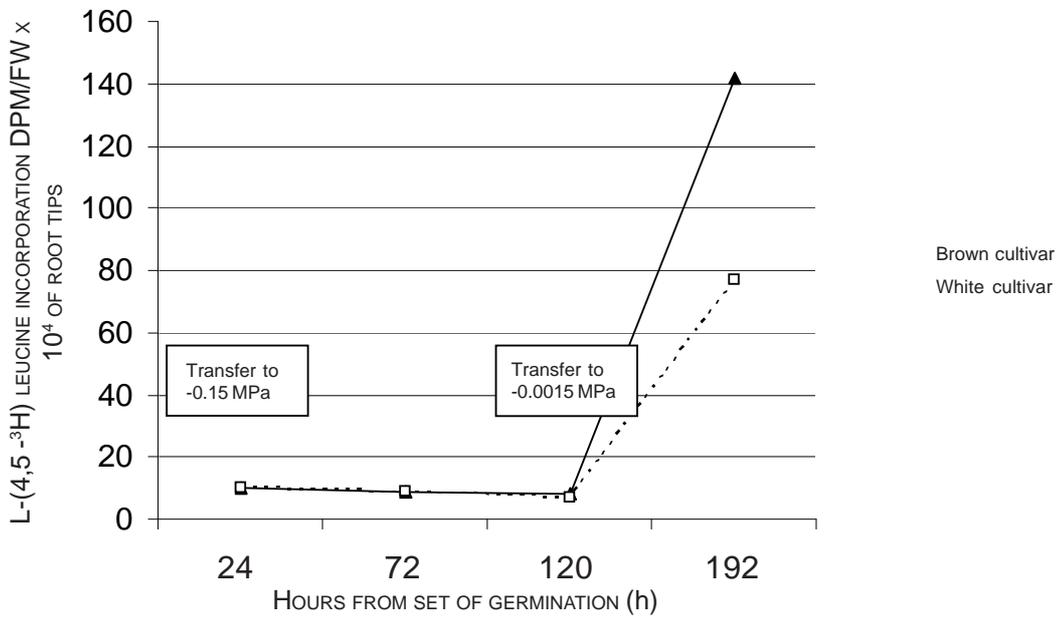


Fig.1a. Incorporation of DPM/FW x 10⁴ of L-[4,5-³H] of leucine into the root tips of white-seeded (□) and brown-seeded (▲) cowpeas after seeds had been exposed to readily water availability (-0.0015 MPa) for 24 h and subjected to water stress (-0.15 MPa) until 120 h before returning to readily water availability up

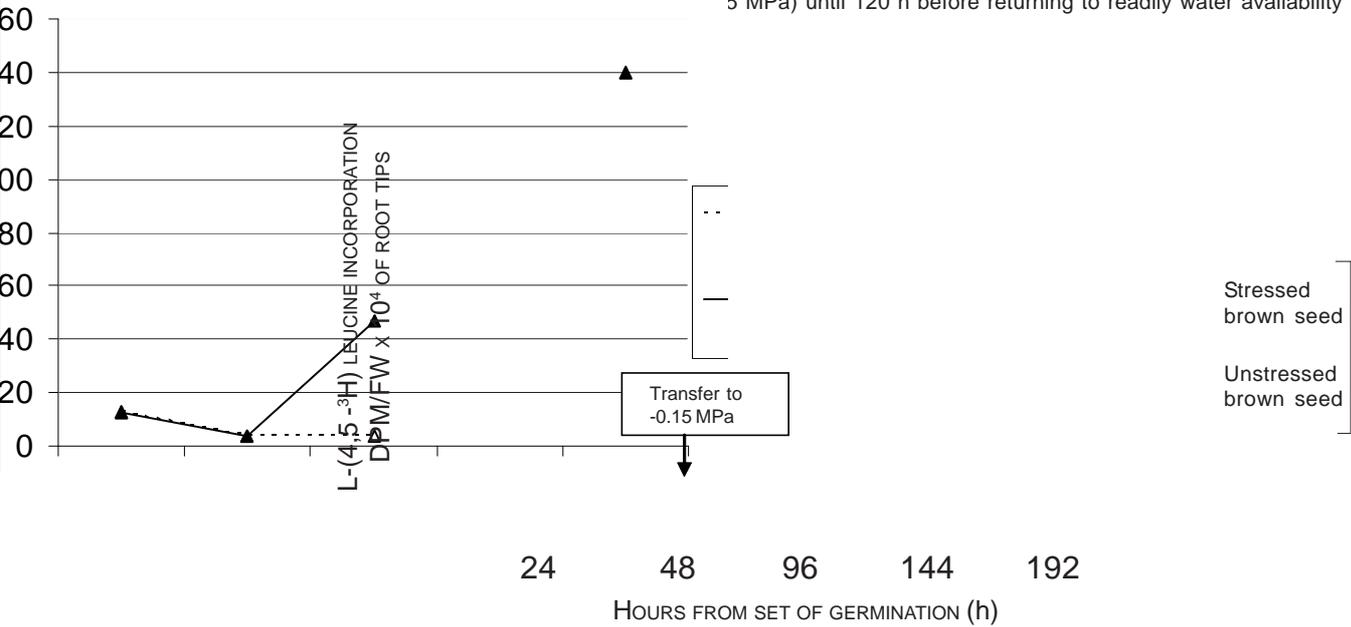


Fig. 1b. Incorporation of DPM/FW x 10⁴ of L-[4,5-³H] of leucine into the root tips of a pigmented cowpea cultivar (TVX 2724-01F) initially exposed to readily water availability (-0.0015 MPa) until 48 h before subjecting one set to water stress (-0.15 MPa; △) and readily water availability (-0.0015 MPa; ▲) up to 96 h.

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