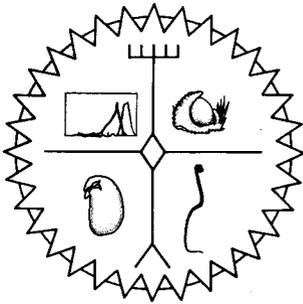


Phytochrome-mediated accumulation of free amino acids in radicles of germinating watermelon seeds

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The total free amino acid pools in radicles of watermelon seeds, investigated during imbibition of water at 25°C, were higher under the most (darkness) than under the least (continuous broad spectrum far-red light) favourable light regime for germination. When seeds were imbibed in an appropriate osmotic solution of PEG-6000 (fully suppressing germination), in darkness or under continuous red or far-red light, the biochemical analyses of the radicles after 1, 2, 3 and 4 days from the onset of imbibition show that while the total soluble sugar content remains rather constant in all treatments, significant changes are observed in the total free amino acid pools. After the first day, a considerable increase characterizes the “darkness” pool in contrast to a moderate one under red, while the “far-red” pool remains constant. Ultimately, at 4 days, the three pools are 190, 142 and 123% of the 0 day radicle one. The qualitative free amino acid determination of the 4 day darkness and far-red pools shows a considerably increased percentage contribution of glutamic acid, arginine and citrulline in the “darkness” pool. The free amino acid increase in non-illuminated radicles may be correlated to germinability; moreover, it is evidently a phytochrome-mediated, pre-germinatory event, probably due to the hydrolysis of proteins (known to be rich in glutamic acid and arginine), stored in the radicle.

Additional key words – *Citrullus lanatus*, germination potential, osmotic inhibition, soluble sugars.

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Introduction

In some cases control of seed germination and dormancy lies in the balance between the restrictive external seed layers and the expansive force of the embryonic axis (and especially of the radicle). In their pioneering work, Scheibe and Lang (1965) reported that red (R) irradiation given to light-sensitive Grand Rapids lettuce achenes increased the growth potential of the embryonic axes. This growth increase was afterwards attributed to the water potential decrease of R-treated embryos (Nabors and Lang 1971a,b). Nabors and Lang (1971b) suggested that germination of Grand Rapids achenes could be the result of R- (and phytochrome-) mediated lowering of osmotic potential in the embryonic axis. The osmotic potential decrease should take place through an

increased enzymatic hydrolysis of storage materials, which in turn would lead to accumulation of osmotically active substances, such as amino acids, soluble sugars and organic acids. This suggestion was reinforced by the microscopical observation of R-induced degradation of protein bodies in Grand Rapids radicles incubated in mannitol solution (Nabors et al. 1974)

Detailed biochemical analyses in embryonic axes of R- and far-red (FR)-treated Grand Rapids did not reveal any significant differences in the osmotic compounds (Carpita et al. 1979b), although a considerable water potential difference was obtained (Carpita et al. 1979a). It was assumed that osmotic and pressure potential changes in the embryonic axes of R-treated achenes are the result of a phytochrome-stimulated proton pump. However, work done in parallel by Takeba (1980a–d)

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with the New York 515 improved cultivar of lettuce gave contradictory results. Takeba (1980a, b) was able to discover a considerable accumulation of free amino acids in the embryonic axis of germinating, non-dormant achenes. He showed that this accumulation is a phytochrome-mediated, pre-germinatory event, also influenced by temperature and hormones (Takeba 1980c, d). Furthermore, by estimating the volume of the radicle tip, Takeba (1980b) suggested that the accumulation of free amino acids can account for the growth potential increase of germinating New York lettuce achenes.

Watermelon [*Citrullus lanatus* (Thunb.) Matsu. et Nakai] seeds are dark-germinating, like seeds from most cultivated species of *Cucurbitaceae*. Their dark germination is nearly 100% in the temperature range 20–40°C. Throughout this range, continuous far-red (cFR) light is inhibitory and germination is 0% at 20–30°C. Continuous red (cR) illumination at 25°C, though not inhibitory to final germination in water, results in lowered germination potential, i.e. in decreased germinability when imbibition occurs in osmotica (C. A. Thanos, 1980. Ph.D. Thesis, Univ. of Athens, Greece). Germination of watermelon seeds has been shown to be under phytochrome control (Loy and Evensen 1979, C. A. Thanos 1980. Thesis). Only continuous or intermittent (but not a brief) FR irradiation can prevent germination and this fact has been attributed to a slow dark transformation of phytochrome intermediates to the active form, P_{fr} (Loy and Evensen 1979, C. A. Thanos 1980. Thesis). Therefore, watermelon seeds were given darkness, cFR and cR (i.e. the best, worst and intermediate light regime for germination, respectively). It was also decided that biochemical analyses would be carried out on radicles (and not on whole embryonic axes), since it was considered more probable for any biochemical changes during germination to be larger there. The location of ninhydrin-positive substances in the radicle tip of New York lettuce (Takeba 1980b) justifies this decision.

Abbreviations – c, continuous; D, darkness; eq., equivalents; FR, far-red; PEG-6000, polyethylene glycol with average molecular weight 6000 dalton; R, red.

Materials and methods

Seeds and experimental conditions

Watermelon (*Citrullus lanatus* [Thunb.] Matsu. et Nakai, cv. Sugar Baby) seeds, 1975 harvest, were purchased from KYΔEII, Greece. The experiments presented here were carried out in 1979. Imbibition and germination tests were performed with 25 seeds per petri dish (diam. 9 cm), each lined with two layers of filter paper moistened with 6 ml of either deionised water or osmotic solution. Throughout the experimentation, temperature was kept constant at 25 ± 1°C. The light sources used have been described previously (Georghiou and Thanos 1983). The criterion of germination was radicle

protrusion; germination values (Fig. 3) are means of 8 replicates. Vertical lines in figures represent SE.

Biochemical procedure

Seeds were imbibed, under different light regimes and for various periods of time, in water or PEG-6000 (30% w/w) purchased from BDH Chemical Ltd (Poole, England). Afterwards, seeds were washed immediately with water and surface-dried. After an additional 24-h-period of air-drying in darkness, seeds were deocoted and radicle tips (about 1 mm long) were cut off with a razor blade. The radicles were grouped in 6 samples of 30 tips, and each sample was weighed and extracted five times with boiling ethanol (80, 80, 50, 50 and 80% v/v, respectively). Final volumes of extracts were adjusted to 15 ml.

Free amino acids and soluble sugars were determined with ninhydrin and orcinol reagents (Kessler 1967), respectively, using a Technicon Auto-Analyser. Since the quantities of the total free amino acids in the 6 samples of each treatment did not differ significantly, the final determination of the individual amino acids was carried out on the combined extracts (mixture of 6 extracts).

Results

Total free amino acids in water imbibition

The average dry weight per radicle was 0.29 mg in all treatments presented in this work, with the only exception of 24 h dark imbibition in water, where it amounted to 0.35 mg. This increase is attributed to transport of materials from the hypocotyl and less probably the cotyledons, and can be explained by both imbibition and germination data (C. A. Thanos, 1980, Ph.D. Thesis, Univ. of Athens, Greece). The second, stationary phase of imbibition kinetics takes place between 20 and 30 h after sowing, while germination starts at around 20 h and is about 5% at 24 h (96% at 41 h). It is therefore clear that the increase in free amino acid concentration in dark-treated radicles presented in Fig. 1 was very rapid. There are statistically significant differences as early as 12 h after onset of imbibition and while free amino acids increased steadily in darkness, they seemed to level off after 18 h under cFR.

Total free amino acids in osmotic imbibition

For separating the final event of germination (radicle protrusion) from the underlying biochemical processes, seeds were imbibed in a solution of PEG-6000 with an estimated water potential around –1.1 MPa (Michel and Kaufmann 1973). This solution was just inhibitory for germination and its water potential value is comparable to the one of inhibitory mannitol solution (–1.25 MPa, Fig. 3). Figure 2 reveals a considerable free amino acid accumulation in dark-treated radicles and a rather weak one under cR. The total free amino acid concentration

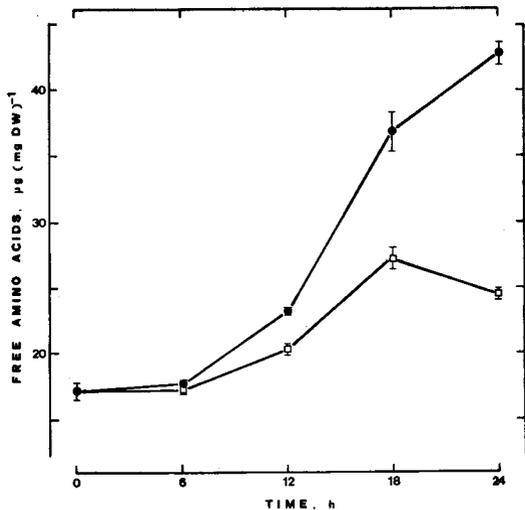


Fig. 1. Time course of free amino acid concentration (Tyr equivalents) in radicles of seeds imbibing in water at 25°C, under D (●) or cFR (□). Bars show SE.

under cFR did not change after the first day, while all differences from 2 days and onwards are statistically significant. The total quantities of free amino acids reached their maximal values at the 4th day, being 190, 142 and 123% of the values for 0 day radicle under darkness, cR and cFR respectively.

The curves presented in Fig. 3 show that the germination potential of watermelon seeds illuminated with cR is

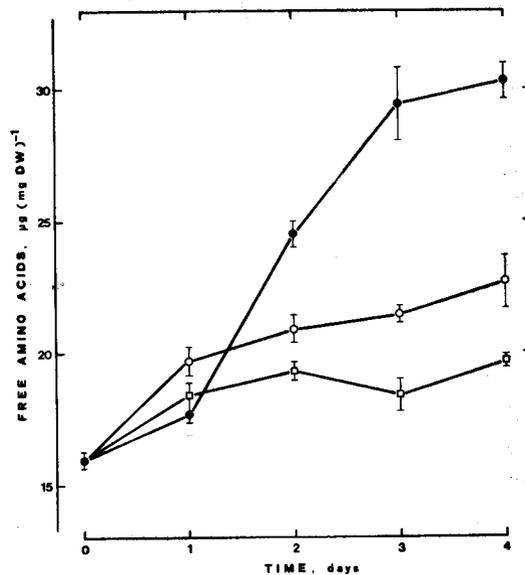


Fig. 2. Time course of free amino acid concentration (Tyr equivalents) in radicles of seeds imbibing in PEG-6000 at 25°C, under D (●), cR (○), or cFR (□). Bars show SE.

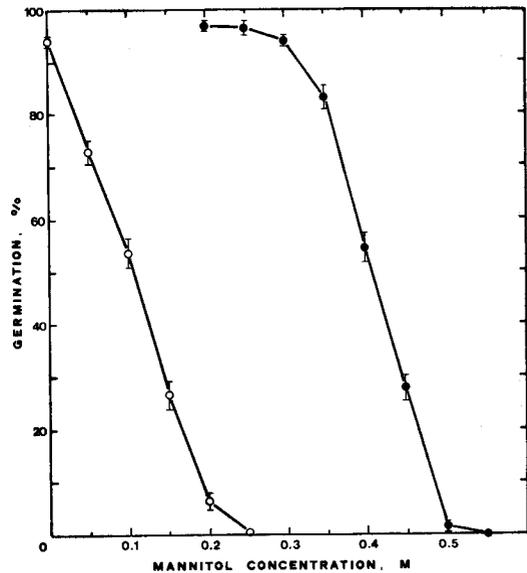


Fig. 3. Germination after 96 h in D (●) or under cR (○) as a function of the molar concentration of the imbibition medium (mannitol solution at 25°C). Bars show SE.

decreased by about 0.30 M or 0.75 MPa, in comparison with those in darkness.

Composition of free amino acid pools

Table 1 presents the molar profiles of free amino acids in the three most extreme treatments of Fig. 2. Ammonia is also included because of its close interrelationship with the amino acids. In the largest fraction of Thr + Ser + Asn + Gln, the amino acid β -pyrazol-1-ylalanine should also be included (Noe and Fowden 1960, Murakoshi et al. 1972). With the exception of glutamic acid, the free amino acid profiles of osmotically treated seeds (D and cFR) share several common characteristics. Thus in comparison with 0 day radicle free amino acids, and apart from the increase of ammonia (especially under cFR), there is a decrease of aspartic acid and increases in arginine and citrulline in cFR and D.

Soluble sugars

The total soluble sugar concentration in the same extracts used in Fig. 1 followed similar kinetics for both treatments, with a slight decrease of 10% (from the 0 h value) at 12 h and a gradual increase afterwards up to a level 10% higher than 0 h. The data presented in Tab. 2 are quite similar to the above mentioned, with fluctuations amounting to approximately 10% of the 0 day value. Nevertheless, it must be noted that the sensitivity of the method shows large variation towards various sugars (Kessler 1967).

Tab. 1. Contribution of individual free amino acids (% on a molar basis) to the total free amino acid pool, in radicles from "dry" seeds and from seeds imbibed for 4 days in PEG-6000 at 25°C under D (darkness) of cFR. (+:trace). *Thr+Ser+Asn+Gln fraction is expressed in Thr eq., Cit in Glu eq., α -NH₃But in Ala eq., and X in Lys eq.

Amino acid	"Dry"	cFR	D
Asp	25.5	10.5	9.9
Thr+Ser+Asn+Gln*	38.2	41.6	36.8
Glu	13.0	10.3	18.6
Cit*	0.8	1.6	3.7
Gly	1.5	+	0.6
Ala	3.6	4.1	4.4
α -NH ₃ But*	0.5	0.4	0.3
Val	1.9	2.3	2.6
Met	+	+	-
Ile	0.5	0.8	0.8
Leu	0.6	0.6	0.6
Tyr	0.7	0.3	0.6
Phe	0.7	0.7	1.1
γ -NH ₃ But	1.5	-	-
NH ₃	5.2	21.1	9.9
Orn	1.0	0.7	0.7
Lys	0.7	0.2	0.4
X*	0.9	0.9	0.4
His	2.8	0.6	1.2
Arg	0.4	3.3	7.4
Pro	-	-	-

Discussion

From the results presented, it is evident that free amino acid accumulation in the radicles of germinating watermelon seeds is a pre-germinatory biochemical event. It is also clear that free amino acid concentration is under phytochrome control and maximal values are observed in darkness, where phytochrome is optimally present and operative (Loy and Evensen 1979, C. A. Thanos, 1980, Ph.D. Thesis, Univ. of Athens, Greece). In a previous study on watermelon (dwarf strain WB-2), Evensen and Loy (1978) did not detect any early differences in free amino acid concentrations in embryonic axes imbibing in water, in darkness or under gold light. This should be principally attributed to the spectral properties of the light used; the "gold" light slightly decreased only the mean time for germination. In addition, the biochemical method used for free amino acid determination is not very refined, although about 100 nmol per axis (in "dry" seeds) were detected, a value comparable to the estimated 50 nmol per radicle of the present work.

Tab. 2. Time course (in days) of soluble sugar concentration [μ g (mg DW)⁻¹, Glc equivalents] in radicles of seeds imbibing in PEG-6000 under D, cR and cFR at 25°C. Mean \pm SE.

	0	1	2	3	4
D	84.2 \pm 1.0	82.3 \pm 1.0	83.9 \pm 1.0	90.0 \pm 1.7	93.4 \pm 0.8
cR	84.2 \pm 1.0	74.6 \pm 1.0	79.6 \pm 1.0	88.4 \pm 2.1	93.9 \pm 0.8
cFR	84.2 \pm 1.0	80.7 \pm 0.9	85.0 \pm 1.4	87.5 \pm 1.1	86.8 \pm 0.8

Free amino acid accumulation may be correlated with germinability and germination potential although the data presented here are not statistically sufficient. Nevertheless, they generally agree with the findings of Takeba, although in New York lettuce considerable accumulation of free amino acids (in water imbibition) was observed rather late, when about 50% of the seeds had already germinated. The soluble sugar concentration in "dry" lettuce axis is 4-fold of the free amino acid one (Takeba 1980b), exactly as in 0 day watermelon radicles. The major point stressed by Takeba (1980b) is that the free amino acid concentration increase can account alone for the total water potential decrease (and the consequent growth potential increase). The application of Takeba's reasoning to these experiments on watermelon seeds gives dubious results. Thus in the case of cR, the free amino acid concentration decrease (in comparison with darkness) accounts only for 0.15 MPa, while the germination potential decrease is about 0.75 MPa. Therefore it might be assumed that either the free amino acid accumulation is located in the extreme end of the radicle tip (with the 1/5 of the total radicle volume) or an additional factor is involved (e.g. a lowered pressure potential, as suggested by Carpita et al. 1979b).

The considerable increase of both arginine and glutamate in the 4 day dark-treated radicles leads to the conclusion that free amino acid accumulation is due to protein hydrolysis. The storage proteins in the seeds of *Cucurbita moschata* (Chou and Splittstoesser 1972) and *Citrullus lanatus* cv. Galaxy (S. Vrettou-Vallianatou, 1981. Ph.D. Thesis, Univ. of Athens, Greece) are especially rich in glutamate + glutamine and arginine (20.7, 13.3% and 18, 12% respectively). Furthermore, the marked increase of citrulline could be attributed to the increased presence of its precursor, arginine (Beevers 1976). The large decrease of aspartate in both D and cFR, as well as the decrease of glutamate in cFR, might be due to their deamination and subsequent respiratory utilisation, which could also explain the particularly high levels of ammonia. Both the rate of accumulation of amino acids (Fig. 1) and the fact that arginine is not usually transported (Beevers 1976), lead to the conclusion that the most probable site of protein hydrolysis is the radicle itself.

A further and deeper investigation of free amino acid accumulation during early stages of seed germination is certainly needed. Such research could offer new and enlightening information on both phytochrome action and the mechanism of germination, as well as on the biochemical processes underlying seed osmoconditioning.

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