Effect of ABA and GA_3 on Protein Mobilization in Embryos and Cotyledons of Angico [*Anadenanthera peregrina* (L.) speg] Seeds During Germination.

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ABSTRACT

In this work, a woody species [A. peregrina (L.) Speg.] *was studied in order to observe the effect of ABA and GA_3 at the biochemical level during the process of seed germination. Embryos incubated in sucrose solution containing ABA and/or GA_3 were analyzed through SDS-PAGE to observe the mobilization pattern of storage proteins during the beginning of germination. Cotyledons isolated from seeds incubated in aqueous solutions containing ABA and/or GA_3, were also analyzed through SDS-PAGE and by PAGE/Activity Gels (polyacrylamide gels copolymerized with substrate for enzymes) to observe the mobilization pattern of storage proteins and protease activity after the beginning of the germination. Results of these experiments show that ABA blocks protein mobilization by inhibiting protease activity in cotyledons. This inhibition is not sufficient to prevent germination showing that the effect of ABA on germination is not dependent on protease activity. The blockage of storage protein mobilization was also observed in embryos, but no protease activity inhibition was clearly detected. ABA was able to induce the synthesis of proteins in cotyledons but not in embryos. A polypeptide with an approximate molecular weight of 17 kD, was degraded within 6 hours in control embryos, but this degradation was blocked by ABA and GA_3. Using the same concentrations of ABA and GA_3 on embryos and cotyledons, the effect of ABA was counteracted by GA_3 in embryos, but not in cotyledons. Although the effects of ABA and GA_3 were not so different from those shown in the literature, the behavior of 17 kD-polypeptide contradicts these reports suggesting that specific studies should be performed.*

Key words: ABA, GA_3, germination, protein pattern, protease activity, tropical woody plant.

INTRODUCTION

Seeds of cultivated species have been used as experimental models in studies of biochemical mechanisms of the germinative process, such as synthesis and mobilization of seed reserves (Fincher, 1989; Shotwell & Larkins, 1989; Shutov & Vaintraub, 1987; Ryan, 1973); characterization of storage proteins (Shewry *et al*, 1995; Barros & Larkins, 1981; Larkins, 1981) or hormonal control of hydrolase synthesis by the aleurone layer of cereals (Nolan & Ho, 1988; Jacobsen & Beach, 1985).

The use of these seeds as experimental models has the convenience of being genetically uniform in the same population. In general, this implies into an uniform germination. However, especifically for this uniformity, Mayer and Shain (1974) suggest that "these species are not ideal models for the comprehension of the mechanisms which control germination as a whole".

Wild species do not present this uniformity. Seeds of wild species which present dormancy are under a complex mechanism of germination control. Polymorphous species that present seeds of different sizes, shapes and mass, can throw seeds in the soil at different stages of development, thus leading dormancy to occur in different degrees of intensity (Mayer & Poljakoff-Mayber, 1989). Dormancy variability implies distinct germinative behaviors that allow the dispersion of germination along with time and space, which in turn prevents the occurrence of mass germination under possible unfavorable

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environmental conditions (Mayer & Poljakoff-Mayber, 1989; Mayer & Shain, 1974).

Among the factors controlling germination, are the endogenous levels of phytohormones mediating alterations of seed physiological and biochemical state which result in resuming the embryo development. These alterations are complex and converge for the activation and synthesis *de novo* of hydrolytic enzymes which break the reserve macromolecules. The products of the hydrolyses are then used for the growth of the embryo axis (Fincher, 1989).

The protein synthesis presents distinct patterns during the germinative process (Oishi & Bewley, 1992) and the phytohormones have a key role in inducing or repressing this synthesis. In studies of the germinative process, emphazis has been given to the influence of gibberellins (GAs) and abscisic acid (ABA), due to the antagonism between them in relation to their promoting and inhibiting effects on germination, respectively (McCarty, 1995; van Beckum *et al*, 1993; Qi *et al*, 1992; Kohler & Ho, 1990; Nolan & Ho, 1988; Jacobsen & Beach, 1985, Schopfer & Plachy, 1985; Higgins *et al* , 1976; Harvey & Oaks, 1974).

It is a concensus that the GAs are the embryo's diffusive factors for the aleurone layer in cereals and that they promote synthesis of hydrolases through this layer during germination. This concensus comes from studies performed with aleurone layer isolated from barley of the Himalaya cultivar. Aleurone of this barley cultivar has been used as an experimental model to study the effects of GA_3. The GA_3 pattern sensitivity is obtained from these aleurone layers, in which in the absence of this growth regulator, little or no α-amylase is synthesized (Fincher, 1989). However, Fincher observes that "other cereals, including other barley varieties, are relatively insensitive to GA_3 and it has not been demonstrated unequivocally that the GAs are diffusive factors in intact grains for all cases" (Fincher, 1989). However, what is known about the protein synthesis induced by GA_3 during germination derives from studies with this model. In the aleurone layers, GA_3 induces the expression of specific genes that codify for hydrolytic enzymes. For example, GA_3 is capable of inducing the mRNAs synthesis of α-amylase (Nolan & Ho, 1988; Jacobsen & Beach, 1985), proteases (Kohler & Ho, 1990; Nolan & Ho, 1988), nucleases, β-glucanases and other hydrolases (Fincher, 1989). Besides inducing *de novo* proteases synthesis, GA_3 also activates proteases secretion (Hammerton & Ho, 1986; Jacobsen & Varner, 1967).

During germination, GAs effects are antagonized by ABA. It is known that ABA controls embryo maturation and prevents precocious germination by regulating gene expression. Its effect is double. First by preventing the expression of specific genes for germination, especially those which codify for hydrolases in the presence of GA_3 (Fincher, 1989, Hammerton & Ho, 1986, Jacobsen & Beach, 1985). Second, by inducing the expression of embryogenic genes which codify for storage protein and for hydrolase-inhibiting proteins (Merlot & Giraudat, 1997; Ingram & Bartels, 1996; Paiva & Kriz, 1994; Holbrook *et al*, 1991; Rivin & Grudt, 1991; Williamson & Quatrano, 1988; Bray & Beachy, 1985; Finkelstein *et al* , 1985; Higgins, 1984).

Qualitative studies on the effects of these growth regulators during germination have used the electrophoresis and its derivations as the main techniques to observe the pattern of protein and mRNAs synthesis induced by these regulators (Paiva & Kriz, 1994; Barros & Larkins, 1990; Borges *et al*, 1990; Nolan & Ho, 1988; Hammerton & Ho, 1986; Asahi *et al*, 1985). In this work, the denaturating SDS-PAGE and non-denaturating PAGE - Activity Gels (polyacrylamide gels copolymerized with substrate for enzymes) systems were used in order to observe the polypeptide profile and protease activity, in embryos as well as in cotyledons of *A. peregrina*.

The choice of *A. peregrina* was due to the importance of woody species in the succession and ecological climax of most earth ecosystems and the little information at the biochemical level on the control mechanisms of germination, especially of tropical species. This species is a dicotyledon leguminous which presents quiescent seeds (orthodox) with germinative capacity higger than 85% and protein content of

36.5% (data non published). These characteristics added to the facility of embryo isolation, distinctly separeted from the cotyledons, make the seed of this species a good model to study, through electrophoretic analysis, the effects of ABA and GA_3 on the mobilization of proteins in embryos and cotyledons during the germinative process.

MATERIAL AND METHODS

Plant material

Mature seeds of *A. peregrina* used in the experiments were obtained from the Laboratory of Forest Seeds at the Federal University of Lavras-UFLA, from a batch collected in September of 1993 and stored at 10^o C.

Embryo Treatment

-Assay for Protein Mobilization during and after the beginning of Germination

In this assay, embryos of non disinfested mature seeds were isolated after being imbibed in distilled water (dH_2O) under room temperature for 5 hours. In order to analyze the effect of ABA and GA_3 on the synthesis and/or protein degradation in embryos during the germinative process, isolated embryos were incubated in the dark at 25^o C during 6, 12, 24 and 48 hours. The incubations were performed in Petri dishes (10 embryos in each) containing two filter papers humidified with 6 ml of 3% sucrose solution with no regulators, or supplemented with 100 μM ABA; 1 μM GA_3 + 100 μM ABA. In the case of the analysis of ABA and GA_3 effects on protein synthesis and/or degradation in embryos after the germinative process had started, isolated embryos were incubated during 6 hours in the dark at 25^o C in Petri dishes (10 embryos in each) containing two filter papers humidified in 6 ml of 3% sucrose solution with no regulators. After this period, the embryos were transfered to 3% sucrose solutions supplemented with 100 μM ABA; 1μM GA_3 or 1 μM GA_3 + 100 μM ABA and incubated under the same conditions during 6, 18 and 42 hours. After the incubation periods, the embryos were stored at -85^o C until the electrophoretic analysis in SDS-PAGE.

Cotyledon Treatment

-Assay for Protein Mobilization and Protease Activity in Cotyledons

In this assay, non disinfested mature seeds were imbibed for 5 hours under room temperature in dH_2O (control) or aqueous solution of 100 μM ABA; 1 μM GA_3 or 1 μM GA_3 + 100 μM ABA. After imbibition, the seeds were germinated in filter paper rolls (20 seeds in each) humidified continuously with the same treatment solutions during 0, 2, 4, 6, 8, 10 and 12 days. The germination conditions were: 8 hours of darkness at 30^o C and 16 hours of light at 20^o C. After this, the cotyledons were isolated and stored at -85^o C until the electrophoretic analysis in SDS-PAGE for total protein or in PAGE-Activity Gels for proteases.

Protein Extraction

For extraction of total proteins, 5 embryos (average fresh weight = 50 mg) were macerated with 1ml of Tris-HCl extraction buffer [Tris-HCl 62.5 mM pH 6.8; 2.3% SDS; 10% glicerol; 5% β-Mercaptoetanol (Laemmli, 1970)]. Cotyledon proteins were extracted from 50 mg of cotyledons macerated in liquid N_2 and 1 ml of the same extraction buffer. The homogenates were microcentrifuged at 16000x *g* during 15 minutes and to the supernatant, 2 μL of bromophenol blue (BFB)(0.5% w/v) was added. Proteases were extracted from 100 mg of cotyledons macerated in liquid N_2 and 1 ml of sodium phosphate 50 mM pH 7.0 buffer. The proteases were extracted during 2 hours at 4^o C under occasional agitation. After extraction, the homogenate was microcentrifuged at 16000x *g* during 30 minutes at 4^o C. Glicerol and BFB up to 10% and 0.05%, respectively, were added to the supernatant.

Electrophoresis

The discontinuous SDS-PAGE system described by Laemmli (1970) was used to observe the total protein profile of embryos and cotyledons. The protocol of electrophoresis in Activity Gels, obtained for proteases in *A. peregrina* cotyledons, was based on Asahi *et al* (1985) and Barros and Larkins (1990).

RESULTS AND DISCUSSION

ABA effect on the Protein Mobilization and Protease Activity during Germination

The electrophoretic profile of total proteins extracted from cotyledons wich seeds were incubated for 12 days in the presence or absence of ABA and/or GA_3, showed a blockage in the degradation of storage proteins in cotyledons from seeds incubated in the presence of ABA. This blockage was maintained until the 8th day after the beginning of the treatment (Figure 1) and after this period, the protein degradation started and was rapidly completed (data not shown).

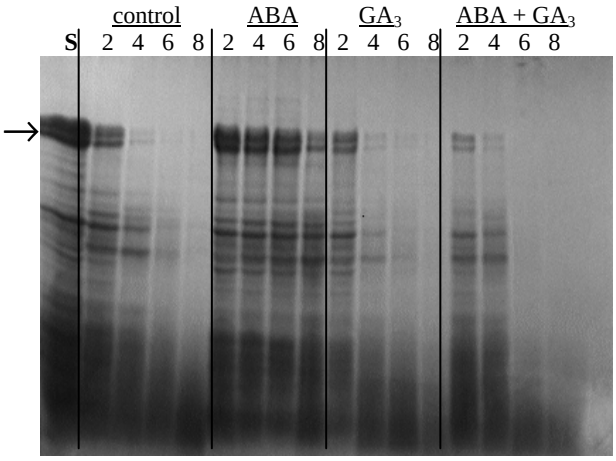

Figure 1 – Protein profile in SDS-PAGE of *A. peregrina* cotyledons isolated from seeds incubated in dH_2O (control), 100 µM ABA, 1 µM GA_3 and 100 µM ABA + 1 µM GA_3 during 2, 4, 6 and 8 days. (**S**), dry seed. **Arrow** = polypeptides with 54 to 66 kD.

The analysis of protease activity in cotyledons shows that it was inhibited until the 8th day in the presence of ABA (Figure 2). The activity started at the 8th day and reached maximum intensity on the 12nd day of incubation. Initial protease activity coincided with the beginning of storage protein degradation. These data indicate that the blockage of protein degradation caused by ABA was due to the inhibition of protease activity. In corn endosperm, the onset of storage protein degradation is attributed to initial protease activity (Barros & Larkins, 1990).

From the results on germination percentage of seeds incubated in the above treatments, it was infered that protease activity and, consequently, the proteolysis of storage proteins were not fundamental for the beginning of germination. In the presence of ABA, 100% of the viable seeds germinated on the 4th day after beginning the treatment, whereas in the control or in GA_3, 100% of the viable seeds germinated on the 2nd day. ABA delayed the germination for 2 days in relation to the control or GA_3. In the presence of ABA, the beginning of protease activity and protein degradation occurred at the 8th day (Figures 2 and 1, respectively), 4 days after 100% of the viable seeds incubated in ABA germinated. In the control or in the presence of GA_3, the beginning of protease activity and protein degradation occurred on the 2nd day of treatment (Figures 2 and 1, respectively), coinciding with 100% germination.

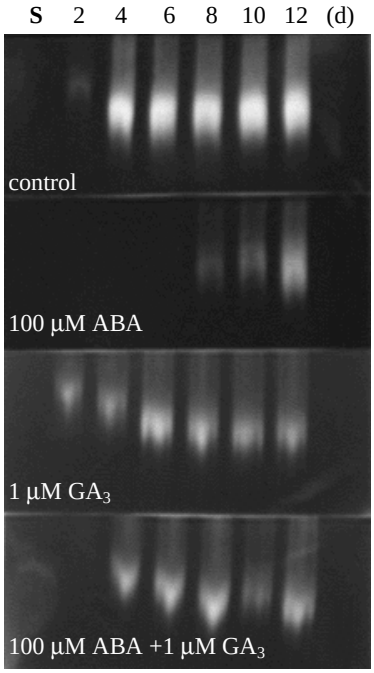

Figure 2 – Activity Gels of proteases extracted from *A. peregrina* cotyledons isolated from seeds incubated during 2, 4, 6, 8, 10 and 12 days (d) in dH_2O (control) or ABA and/or GA_3. (**S)**, dry seed.

It is possible that the aminoacids translocated for protein synthesis, necessary for the initial development of the embryonic axis, were stored in dry seeds (Callis, 1995). The results with *A. peregrina* suggest that ABA has a double effect on this species, affecting germination and proteolytic activity independently. The germination could have been affected by a decrease in the cell wall extensibility and/or due to a disorganization of the actin filaments which, consequently, prevented cell division. Schopfer and Plachy (1985) analyzed the beginning of root development in *Brassica napus* embryos

and they showed that increase in cell wall extensibility was the preponderant factor for water absorption by the cell. These analysis showed that ABA blocks this phase by inhibiting cell wall extensibility without affecting osmotic and turgor pressures. However, ABA affects membrane channels in guard cells by way of promoting a liquid efflux of ions, especially K^+ (Leung & Giraudat, 1998). It reduces the osmotic pressure with consequent water loss by the cell, decreasing cell turgidity and causing the closure of stomatic pore. Analyzing Schopfer and Plachy's (1985) results in relation to the effect of ABA in the membrane and ion channels, Hetherington and Quatrano (1991) suggest that the importance of the wall effect in response to ABA, as much as gene expression or ion flow, varies from one tissue to another. Recent data on stomatic movement suggest that the molecular and cytoplasmic organization of actin filaments in guard cells has a movement shaping function (Leun & Youngsook, 1997; Hwang *et al*, 1997). With the guard cells being turgid, the filaments are polymerized and lay radially in the cells. During induction of stomatic closure by exposure to dark or ABA, the actin filaments depolymerize and randomly occupy a cortical position adjacent to the pore. Chemical agents that depolymerize actin filaments cause the same effect of ABA or exposure to dark (Eun & Youngsook, 1997), including efflux of K^+ (Hwang *et al*, 1997). It suggests that actin filaments are part of the signal transduction in guard cells. However, it is still unknown with what intensity or even if the actin filaments are affected by ABA during germination (which could diminish the preponderance of cell wall extensibility on germination). Therefore, considering the differentiation degree among embryonic and guard cells, it seems plausible to suppose that the opposing alterations in the turgor state between these cells in response to ABA are consequence of different controlling factors or different degrees of intensity of physiological response of each cell type.

The release of germination after 2 days in relation to the control and GA_3 can be related to a "lag" phase, suggested by van Beckum *et al* (1993), in which the embryo reprograms to germinate after "release" of the endogenous ABA. However, the barley embryos with which these researchers worked, "released" to germinate after 2 days, were continuously maintained in the presence of ABA, as in our assay. However, ABA is metabolized by the cell and a *continuum* of its endogenous concentration could have been established (Zeevaart & Creelman, 1988) and suppressed by a possible gibberellin synthesis by the embryo (Fincher, 1989). Thus, it seems that the protease activity was directly affected by ABA, in whatever regulatory level, independently from the germination delay.

In embryos, the results indicated a blockage in the storage protein degradation by ABA (Figure 3). Protease activity in embryos was detected in 24 hours only in the control (data not shown); however, the storage protein degradation was blocked in 6 hours by ABA. It is possible that the activity not observed in the initial hours is related to the detection method and to the lower amount of protein in embryos compared to cotyledons. In a relative disagreement with our data, Borges *et al* (1990) found no difference on the protein profile of *Piptadenia peregrina* embryos, an *A. peregrina* synonym (Lewis, 1987; Allen & Allen, 1981; Cronquist, 1981), treated with ABA in various concentrations up to 12 hours. An increase in the percentage of seed germination was also observed. However, these researchers isolated embryos after seeds were incubated in the presence of ABA and, according to Mayer and Shain (1974), embryo responses to regulators can be diverse between whole seeds and isolated embryos. The increase in germination percentage observed by Borges *et al* (1990) could be related to the low ABA concentration used (1μM). Zeevaart and Creelman (1988) reffers to stimulation of root growth by low ABA concentrations as a consequence of plant adaptation to water deficiency, made possible by ABA.

Balance between ABA and GA_3

According to the results of SDS-PAGE of embryo proteins, GA_3 did not inhibit the ABA effect, as observed in the protein profile between embryos under ABA and ABA + GA_3 treatments (Figure 3), which showed a blockage in storage protein degradation in both treatments. However, the results show that degradation of a polypeptide with molecular weight of aproximately 17 kD was also partially blocked by GA_3 (Figure 3). This polypeptide, an albumin in our classification (data not shown), present in dry embryos, was degraded in 6 hours in the control embryos. In the presence of ABA

and/or GA_3, this degradation was blocked in 6 hours (Figure 4). If this polypeptide was a possible member of a storage protein, GA_3 should not have suppressed its degradation as occurred in the presence of ABA. Nolan and Ho (1988), working with barley aleurone, found mRNAs which have their transcription suppressed by GA_3 and induced by ABA, and they suggest that the inhibition of certain genes by gibberellins is part of the action mechanism of this regulator during the induction of germination. Van Beckum *et al* (1993) observed that a gene responsive to ABA (*Rab*) is not suppressed by GA_3, and they suggest that this gene is not part of the germinative process.

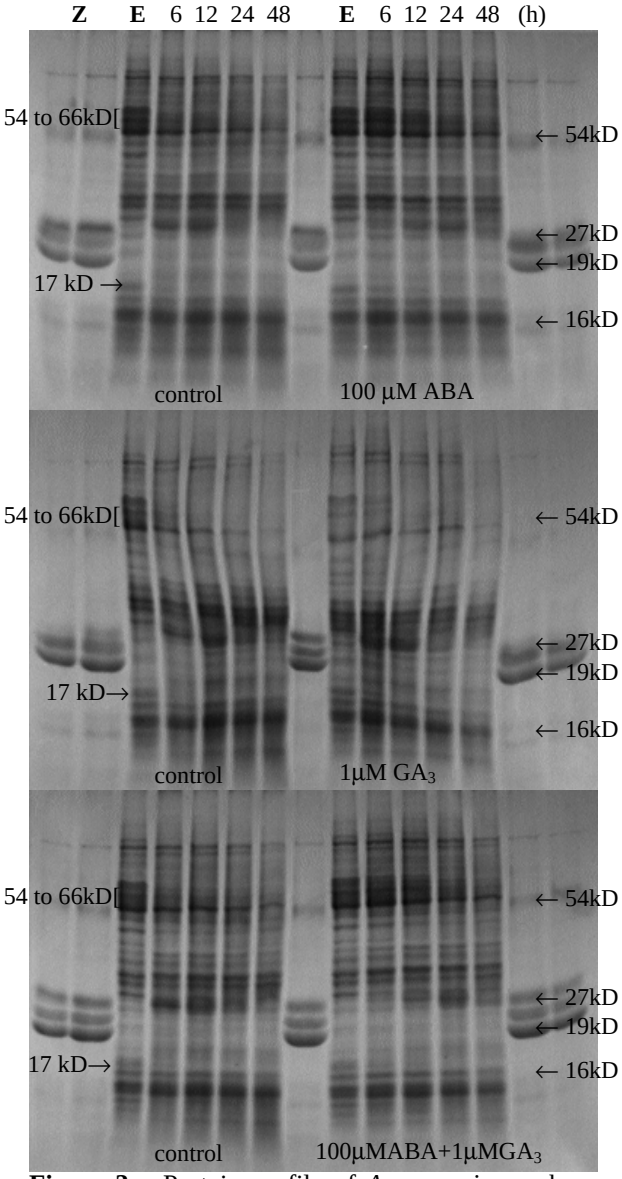

Figure 3 – Protein profile of *A. peregrina* embryos isolated from seeds imbibed in dH_2O during 5 hours (**E**) and incubated during 6, 12 , 24 or 48 hours (h) in 3% sucrose solutions supplemented with or without (control) ABA and/or GA_3. (**Z**), maize prolamins (zeins) used as molecular weight marker.

However, it seems contradictory that the non degradation of the 17 kD polypeptide induced by GA_3 is related, for example, to the inhibition of a protease since GA_3 apparently did not block protease activity in cotyledons (Figure 2), or to the non inhibition of a *Rab* gene once that the polypeptide is already present in dry embryo, degraded in the absence of ABA and probably it is not synthetized through ABA induction (Figure 5).

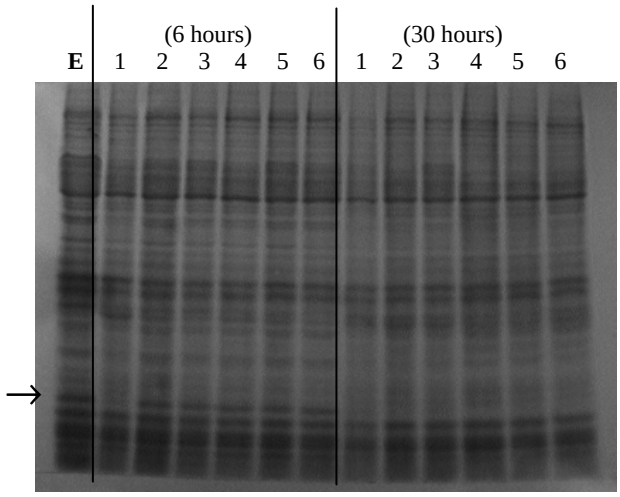

Figure 4 – Protein profile of *A. peregrina* embryos isolated from seeds imbibed in dH_2O during 5 hours (**E**) and incubated in 3% sucrose solutions supplemented with or without ABA and/or GA_3 during 6 or 30 hours. 1) control, 2) 10 µM ABA, 3) 100 µM ABA, 4) 1 µM GA_3, 5) 10 µM ABA + 1 µM GA_3 and 6) 100 µM ABA + 1 µM GA_3. **Arrow** indicates the 17kD-polypeptide.

In cotyledons, the effects were inverse of those observed in embryos. For the same concentration of ABA and GA_3 used in embryos, GA_3 inhibited ABA effect. Degradation of storage protein occurred in a similar way in the control as well as for embryos treated with GA_3 or ABA + GA_3 (Figure 1). Again, the degradation seems to be related to protease activity which was inhibited by ABA, not inhibited by GA_3 and partially inhibited by ABA + GA_3 (Figure 2). Protease activity was observed in barley aleurone treated with 1µM GA_3 or 1µM GA_3 + 100 µM ABA (Hammerton & Ho, 1986). There are no specific data in the literature to explain this inversion observed in *A. peregrina*. However, Torrent *et al* (1989)

showed that in corn there is a degradation of endosperm protein without the embryo and they suggest the possibility of the endosperm from mature seeds contain an accumulation of gibberellins capable of inducing protease synthesis. According to Graebe (1987) inmature seeds usually have gibberellin levels not found in any other plant organ. Nowadays, genes from the biosynthetic route of GAs have been cloned, which are expressed along seeds' development (Hedden & Kamiya, 1997; van Huizen *et al*, 1997). In *A. peregrina*, it is suggested that the addition of GA_3 increased the concentration of endogenous gibberellins in a way that suppressed ABA action. According to Jacobsen & Beach (1985), an ABA excess 25 times larger than GA_3 inhibits the accumulation of α-amylase during 48 hours. After this period, this inhibition by the ABA stops.

Control of Protein Synthesis by ABA

According to the results of SDS-PAGE with embryos, all polypeptides studied were under proteolytic process and none were synthesized, in the presence or absence of ABA and/or GA_3. The addition of ABA after the beginning of germination neither prevent the ongoing proteolytic process, nor induced protein synthesis (Figure 5). It could be related to the incapacity of ABA of inducing dormancy (Khan & Andreoli, 1992). However, it is possible that more sensitive analysis, such as two-dimensional electrophoresis, could reveal a polypeptide member of globulins (Paiva & Kriz, 1994). It is also possible that the embryonic tissue of mature, non-dormant *A. peregrina* seeds have reduced sensitivity to ABA after the beginning of germination. Mature embryos of alfalfa (Xu & Bewley, 1991) and *B. Napus* (Finkelstein *et al*, 1985) have complete insensitivity to ABA, measured in relation to increasing ABA dosages that inhibit germination as well as in relation to the synthesis of specific proteins. In corn, ABA inhibits germination of mature embryos (Rivin & Grudt, 1991), but mRNAs for globulins were detected in embryos incubated in ABA after the beginning of germination (Paiva & Kriz, 1994). The fact that *A. peregrina* has non-dormant seeds, could also be related to its insensitivity to ABA. Van Beckum *et al* (1993) studied the germination of isogenic embryos from dormant and non-dormant barley seeds, both acquired through maturation of seeds from the same line, under conditions of short and long day, respectively. Their studies show that the inhibition of germination by ABA depends on the concentration of exogenous ABA and that embryos from dormant seeds are more sensitive to ABA, as far as germination inhibition, than embryos from non-dormant seeds.

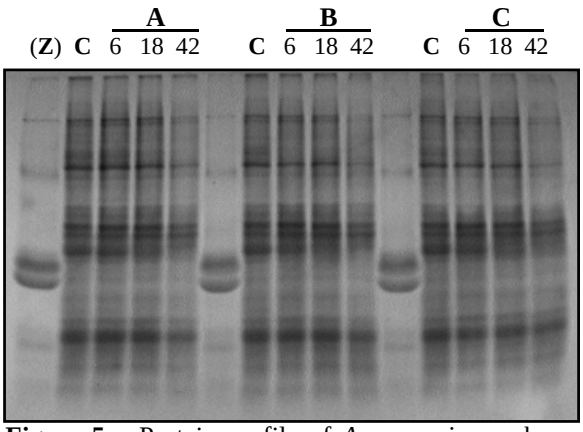

Figure 5 – Protein profile of *A. peregrina* embryos isolated from seeds imbibed in dH_2O during 5 hours and incubated in 3% sucrose solutions during 6 hours (**C**), then transferred and incubated during 6, 18 and 42 hours in 3% sucrose solutions supplemented with **A**- 100 μM ABA, **B**- 1μM GA_3 and **C**- 1μM GA_3 + 100 μM ABA. (**Z**), maize prolamin (zeins) used as a molecular weight marker.

However, in *A. peregrina* cotyledons under ABA treatment, the presence of a polypeptide group with molecular weight higher than 66 kD was observed until the 6th day of incubation (Figure 1), after which they disappeared coinciding with the beginning of protease activity. The results show that some of these polypeptides were present with low intensity in dry seed, and in the presence of ABA, an increase in intensity of these polypeptides occurred, as well as the appearance of new polypeptides. This result is in agreement with the literature as to the ABA's capacity of inducing protein synthesis, already mentioned in this study. However, the nature of these polypeptides was not determined, whether they

are part of storage globulins, as in other dicotyledons, or part of protease inhibitors.

CONCLUSIONS

The results obtained with *A. peregrina* reveal the importance of studing wild species, at least where the antagonism between ABA and GA_3 in controlling germination is concerned. The degradation of the 17 kD-polypeptide shows that the effects of these regulators could diverge from what is reported for cultivated species. However, this is an isolated result and more specific analysis should be performed in order to confirm if this is a direct relation. As to the protein mobilization as a whole, the data obtained do not markedly diverge from the available data in literature, although it was not clearly shown in embryos that the blockage of proteolytic activity is related to the blockage of enzyme activity, as shown in cotyledons.

RESUMO

Controle da Mobilização de Proteínas pelo ABA e GA_3 em Sementes de Angico [*Anadenanthera peregrina* (L.) Speg.] durante a Germinação.

Estudou-se a germinação de uma espécie selvagem sob influência do ABA e GA_3. Embriões isolados, incubados em soluções de sacarose contendo ABA e/ou GA_3, foram analizados em SDS-PAGE para observar o perfil da mobilização de proteínas de reserva durante o início da germinação. Cotilédones de sementes incubadas em soluções aquosas de ABA e/ou GA_3 foram analizados em SDS-PAGE e PAGE/Géis de Atividade para observar o perfil da mobilização de proteínas de reserva e atividade de proteases, respectivamente, após o início da germinação. Os resultados indicam que ABA bloqueia a mobilização protéica através da inibição da atividade enzimática em cotilédones, mas não impede totalmente a germinação, parecendo afetar germinação e atividade enzimática independentemente. Em embriões houve bloqueio da mobilização, mas a relação com a inibição da atividade enzimática não foi claramente demonstrada. ABA induziu a síntese de proteínas em cotilédones, mas não em embriões. Um polipeptídeo com 17 kD é degradado em 6 horas nos embriões, mas a degradação é bloqueada por ABA e/ou GA_3. Para mesmas concentrações de ABA e GA_3, GA_3 não inibiu o efeito do ABA em embriões, mas inibiu em cotilédones. Os efeitos do ABA e GA_3 não diferiram sensivelmente dos dados correntes, mas o comportamento do polipeptídeo de 17 kD é contraditório e sugere estudos específicos.

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