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Full Length Research Paper

Effects of plant hormones and 20-hydroxyecdysone on tomato (*Lycopersicum esculentum*) seed germination and seedlings growth

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20-hydroxyecdysone (20E) is the major phytoecdysteroid of about 6% of plants. Its role in plant physiology has not been fully elucidated. In this work we studied the effects of 20E application on some morphological and biochemical parameters of tomato, *Lycopersicum esculentum*, seed during germination and seedling development (5 d). We compared the 20E effects with the action of phytohormones: gibberellic acid (GA_3), naftalen acetic acid (NAA), benzyl amino purine (BAP). NAA treatment resulted in marked reduction in shoot length. GA_3 treatment promoted maximal shoot elongation. BAP affected negatively shoot length only at late stages, while 20E application stimulated shoot elongation at early stages and reduced it on the fifth day; NAA inhibited root elongation all along the test period. GA_3 treatment had no effect on root length, whereas BAP showed strong inhibition on root elongation. On the other hand, 20E showed a weak inhibition of root elongation on the fifth day. As compared to control and to other treatments, NAA and 20E provoked a drastic decrease in protein contents during seedling growth, whereas a high increase was observed under BAP treatment. Electrophoresis revealed that protein bands were not degraded and mobilized after NAA treatment while in control or after 20E and others phytohormones applications, protein patters displayed weak band intensities and some of them were not detected. NAA, GA_3 and BAP provoked a decrease in proline content during seedlings, while the effect of 20E on proline levels varied during germination and plantlet development. This work showed that 20E like phytohormones fulfil some bioactive actions during germination and seedlings growth in tomato.

Key words: Benzyl amino purine, gibberellic acid, 20-hydroxyecdysone, germination, *Lycopersicum esculentum* seeds, naftalen acetic acid, proline, protein pattern.

INTRODUCTION

Germination of seeds is initiated by imbibition followed by radicle emergence and growth of root and shoot as a result of high metabolic activity (Doganlar et al., 2000). In germinating seeds, storage proteins are hydrolysed and amino acids are released (Lea and Joy, 1983; Gumilevskaya et al., 2001) such as proline and arginin that may be catabolised during the early stages of germination to afford energy to the new formed tissue (Below et al., 2000; Nakashima et al., 1998; Goldraij and Polacco, 1999, 2000). Proline is known to increase during seed germination (Lea and Joy, 1983) and to take part in structural proteins that participate in the edification of cell wall of young tissues (Hare and Cress, 1997; Nanjo et al., 1999).

Plant hormones are a group of organic substances which influence physiological processes mainly growth, differentiation and development (Philosoph-Hadas et al., 2005; Kucera et al., 2005). Cytokinins (CKs) and gibberellins (GA_s) are found in actively dividing tissues of seeds; they are important to breaking dormancy after seed imbibition and allowing germination and growth of dormant embryos (Sondheimer and Galson, 1966; Siob-

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han and McCourt, 2003); GAs promotes synthesis of enzymes that convert stored nutrients as starch to sugars needed for rapid cell respiration during germination, however, abscisic acid (ABA) can counter this effect by keeping seeds dormancy. It is well known that GAs generally have stimulative effects and ABA have inhibitory effects on germination process (Fincher, 1989; Debeaujon and Koornneef, 2000). Oishi and Bewley (1990) demonstrated that ABA affect germination by blocking the induction of α-amylase in cereal aleurone. The same results were obtained with *Phaseolus vulgaris* seeds (Van Onckelen et al., 1980). Garciarrubio et al. 1997 studied the relation between ABA and reserve mobilisation during seed germination in *Arabidopsis thaliana* and concluded that this plant hormone inhibited the germination process by preventing the degradation of the seed storage proteins; the metabolites sugars and amino acids relieved the inhibitory effect.

20-hydroxyecdysone (20E) is the most commonly occurring and the most abundant phytoecdysteroids produced by plants (Lafont, 1997; Dinan, 2001). About 5 - 6% of plants species contain high amounts of phytoecdysteroids (Dinan, 1998). 20-hydroxyecdysone is also the steroidal hormone of arthropods and many other invertebrates where it regulates several physiological processes (Koolman, 1989). In plants phytoecdysteroids are generally considered as secondary metabolism compounds which believed to protect plants against phytophagous insects (Bergamasco and Horn, 1985; Schmelz et al., 1998; Schmelz et al., 1999; Adler and Grebenok, 1999) and nematodes attacks (Soriano et al., 2004). The role of 20E in plant physiology including seed germination is not studied.

The present investigation monitored changes caused by some phytohormones treatments (NAA, BAP, GA_3, and 20E) on germination percentage, shoot and roots initiation and seedlings growth as well as proteins and proline contents in tomato seeds chosen as a model for study because of its easy seed germination and because it is ranked among the most important crops throughout the world (Frusciante et al., 2000).

MATERIALS AND METHODS

Plant Material

Washed seeds of tomato *Lycopersicum esculentum* were surface sterilized during 30 min with 5% (v/v) bleach. Seeds were thoroughly rinsed with deionized water. In a petri dish used for bioassay for germination evaluation of tomato seeds and plantlet shoot and root lenght, 20 seeds were made to germinate on blotter paper soaked with either deionized water or plant hormones (purchased from Sigma, France) or 20 E (a gift of Prof. R. Lafont, Université Paris) and maintained in a growth chamber in darkness at 25°C. Two concentrations (10^{-4} M and 10^{-5} M) of each were replicated three times. Germination percentage was determined. The application of treatment was run out at germinating (planting) time. Germination was determined as the time of radicle emergence. At various stages of the germination process (0, 3, 4 and 5 d), seeds of each replicate were collected for germination percentage. They were stored at –20°C for biochemical analysis.

Proteins measurement

1 g of plantlets was homogenized in 0,1M Tris-HCl, pH 7.2, then centrifuged at 8000 x g for 20 min at 4°C; the supernatants obtained were used for determination of soluble protein content. Protein concentrations were estimated by the Bradford assay (Bradford, 1976) using bovine serum albumin (BSA) as standard

Gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a vertical slab gel apparatus as described by Laemmli (1970). Samples consisting on dry seeds (S) or total plantlets homogenized in 0.2 M NaCl were centrifuged during 20 min at 8 000 x g and the pellet was separated from the supernatant; then both fractions were denaturated by boiling in an equal volume of SDS buffer (2% SDS in 0.0625 M Tris-HC1 10% glycerol 5% 3-mercapto-ethanol pH 7) and run on a 12% acrylamide gel in 0.4 M Tris-HCl buffer at pH 8.8, with constant current set at 20 mA/gel. The gels were stained with Coomassie blue, destained in acetic acid-methanol, and scanned.

Proline measurement

Proline was quantified as described in Bates et al. (1973) with some modifications. Samples (1 g) from different treatments and control were homogenized in 1 ml 3% aqueous sulphosalicylic acid and centrifuged at 1 000 x g. One ml of supernatant was reacted with 1 ml ninhydrin acid and 1 ml glacial acetic acid in a test tube for 60 min at 100°C. The reaction was stopped in an ice bath. The reaction mixture was extracted with 4 ml toluene and mixed vigorously with a test-tube stirrer for 15 s. The chromophore-containing toluene was separated, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using Statistica Software (Statistica, 1997). Post hoc testing was carried out using the Turkey test. A significance level of 0.05 was used for all statistical tests.

RESULTS

Effect of plant hormones and 20E on germination

The test period of germination and postgerminative growth was up to 5 days. After 3 days, tomato seed coat was already ruptured and visible protrusion of radicle indicated the onset of embryonic axis elongation. Treatments were used at either 10^{-4} M or 10^{-5} M in all experiments.

The results of Figure 1A showed that after 3 d period, treatment with NAA at concentration of 10^{-4} M showed no significant changes compared to control, while germination rate under GA_3, BAP and 20E were 90, 78.5 and 54%, respectively (Figure 1A); later on, NAA slightly promoted germination from day 4 as well as BAP treat-

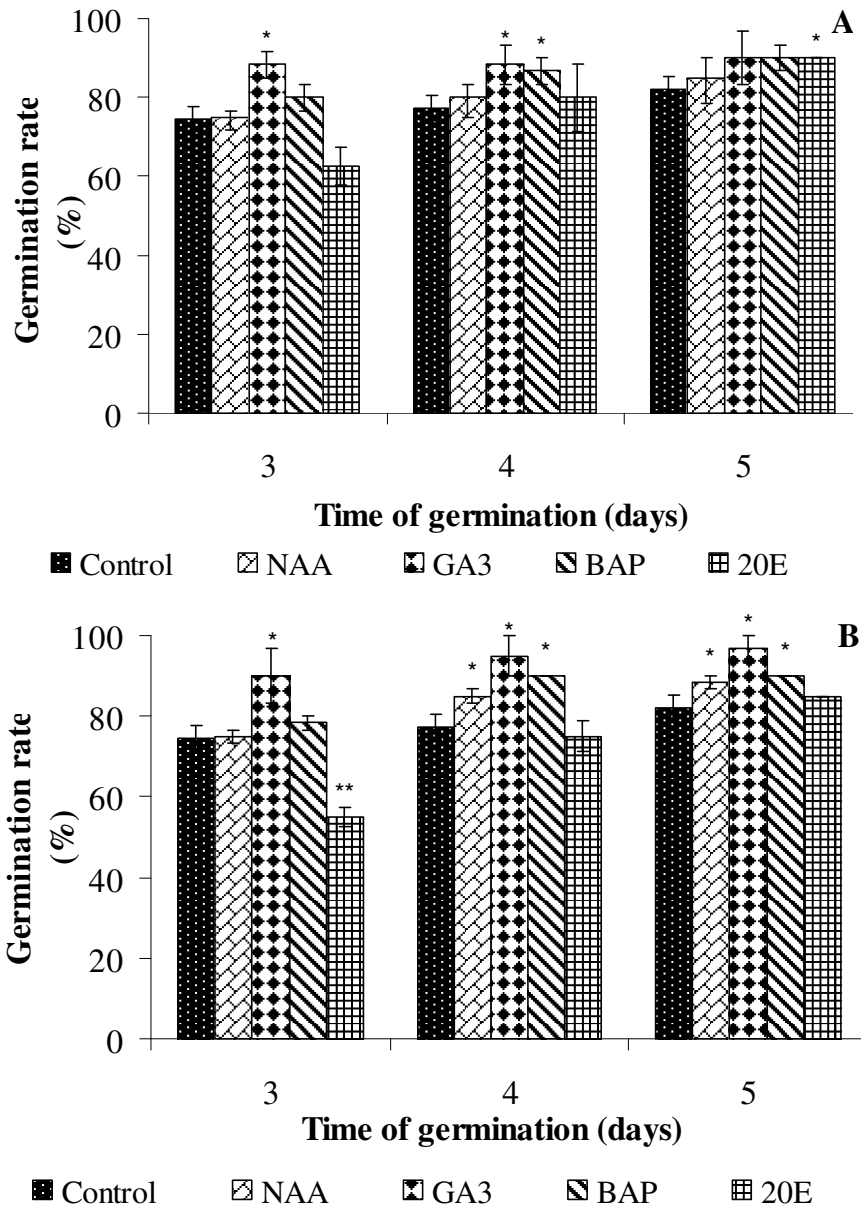

Figure 1. The effect of plant hormones and 20-hydroxyecdysone on germination percentage of tomato seeds. Each value represents the mean + SE of 3 replications. **(A)**: treatment at 10^{-4} M. **(B)**: Treatment at 10^{-5} M. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively. NAA: acid naftalene acetic; BAP: benzyl amino purine; GA_3: gibberellic acid; 20E: 20-hydroxyecdysone.

ment. Thus, 20 E was the only treatment which inhibited germination at day 3 then this inhibitory effect was overcome 24 h later to reach the control level. On the other hand, Figure 1B dealing with the concentration of 10^{-5} M showed that GA_3 and BAP treatments had a slight stimulatory effect on germination percentage; GA_3 stimulated germination from day 3 and BAP did it later from day 4. Application of NAA induced very small differences in emergence. However, 20E slightly inhibited germination after 3 days of germination, showing a percentage of 62.5% compared to 75% in the control then reached the control values after what it slightly stimulated it to be at the other plant hormone effect at the last day of experiment.

Effect of plant hormones and 20E on seedlings development

For this purpose, experiments were conducted with seeds germinating in water, plant hormones or 20E in order to measure shoot and root elongation during 5 d.

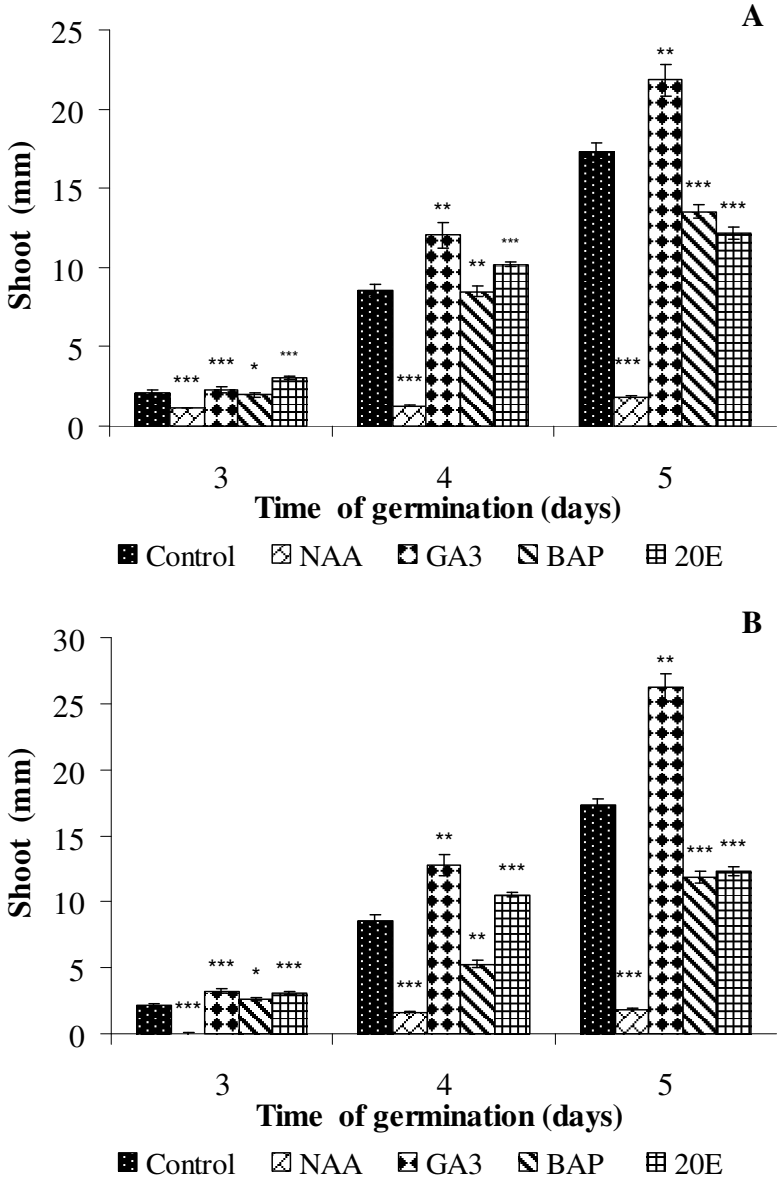

Figure 2. The effect of plant hormones and 20-hydroxyecdysone on shoot length of tomato seedlings. Each value represents the mean + SE of 3 replications. **(A)**: treatment at 10^{-4} M. (**B**): Treatment at 10^{-5} M. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively NAA: acid naftalen acetic; BAP: benzyl amino purine; GA_3: gibberellic acid; 20E: 20-hydroxyecdysone.

The development of tomato seedlings responded differently to the various treatments. Results of NAA treatment showed very strong inhibition of shoot elongation. This inhibition was maintained all over the test time at both concentrations used (Figures 2A and B). No substantial change in the rate of shoot elongation was observed under BAP treatment at early stage, but an inhibitory effect on shoot elongation at late stages was displayed; maximum stimulation of shoot elongation was observed all over the test period under GA_3 treatment. The promotive effects of GA_3 were concentration dependent (Figures 2A and B). The elongation rate increased concomitantly with time; it was 6 fold in presence of GA_3 at concentration of 10^{-5} M in comparison to 4 fold in control from day 3 to day 4. The same results were obtained for both concentration used when 20E was used. It stimulated shoot elongation process during the 4 first days then the rate was highly reduced (12.33 mm) than that of the control (17.28 mm) at the last day of the test (Figure 2A).

For root elongation measurements, seed treatment with NAA resulted in a sharp inhibition of root elongation all

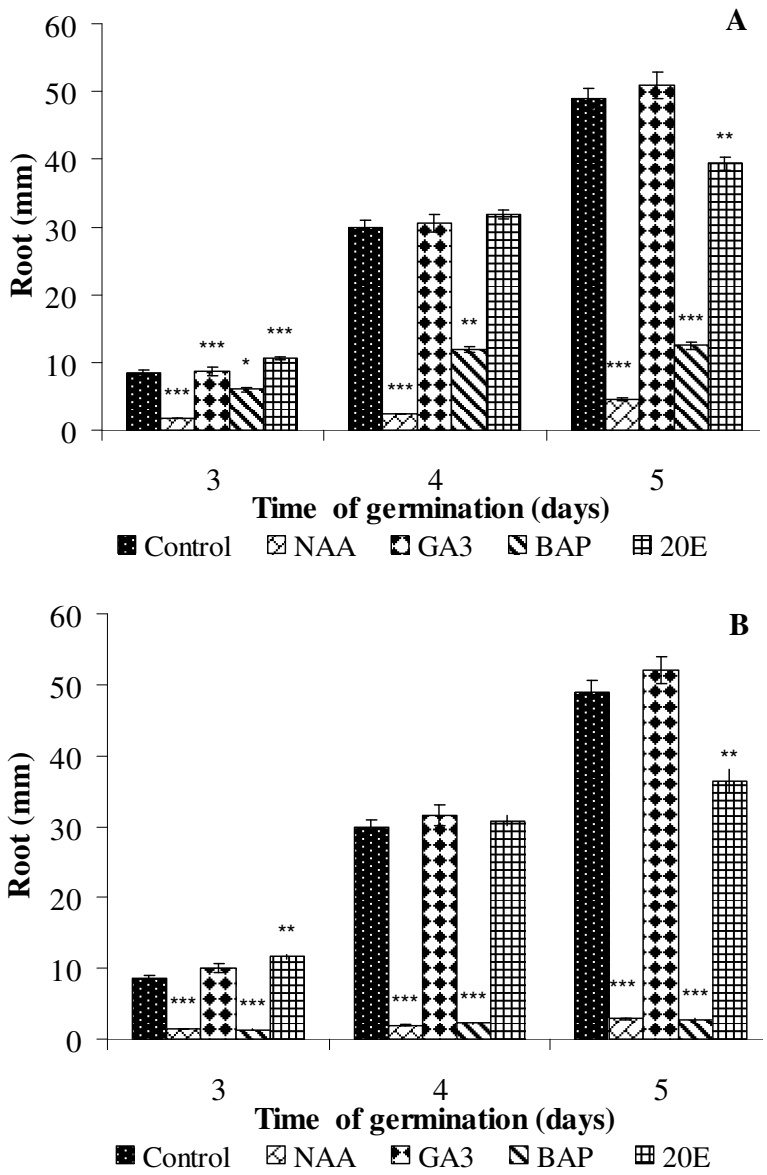

Figure 3. The effect of plant hormones and 20-hydroxyecdysone on root length of tomato seedlings. Each value represents the mean + SE of 3 replications. **(A)**: treatment at 10^{-4} M. (**B**): Treatment at 10^{-5} M**.**
*, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively. NAA: acid naftalen acetic; BAP: benzyl amino purine; GA_3: gibberellic acid; 20E: 20-hydroxyecdysone.

along the test period for both concentrations used (Figures 3A and B). Even by using any concentration, GA_3 treatment had no effect on root elongation. However, BAP showed strong inhibition on root elongation with 10^{-5} M (Figure 3B) which was concomitantly with time of exposure (12 and 12.5 mm compared to 30 and 49 mm in the control at day 4 and 5 respectively) and similarly to NAA at concentration of 10^{-4} M (Figure 3A). On the other hand, different doses of 20E (10^{-4} M, 10^{-4} M) showed no effect at early stages but ended with inhibition. The root length reached 36.47 mm at 10^{-4} M and 39.5 mm at 10^{-5} M compared to control 49 mm for the 5-day-old seedlings.

Effect of treatments on protein content

Figure 4 displayed that proteins decreased during germination process and seedlings growth. In presence of NAA, no effect was detected in either concentration used; whereas the protein contents with GA_3 treatment at a concentration of 10^{-4} M decreased sharply with largest

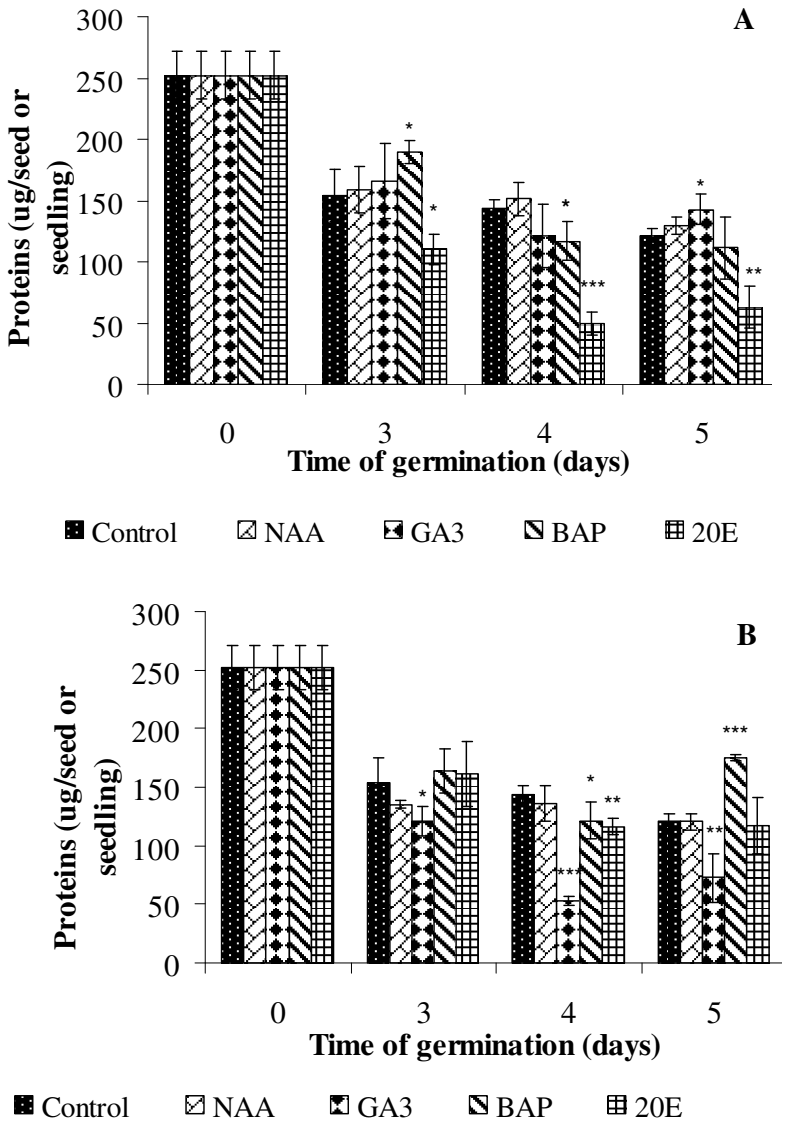

Figure 4. The effect of plant hormones and 20-hydroxyecdysone on proteins content in seedlings from germinating tomato seeds. Each value represents the mean + SE of 3 replications. (**B)**: Treatment at 10^{-5} M. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively. NAA: acid naftalene acetic; BAP: benzyl amino purine; GA_3: gibberellic acid; 20E: 20-hydroxyecdysone.

changes occurring in the 4th and the 5th day and showing a drop to 52.8 and 73 µg/seed compared to untreated seeds which contains 143 and 120.76 µg/seed or seedling respectively (Figure 4A); however it showed a slight increase that reached 142.9 µg/seed or seedling compared to control 120.8 in 5-days-old seedlings at the concentration of 10^{-5} M (Figure 4B). In presence of BAP treatment, at a concentration of 10^{-5} M, a slight increase at day 3 was observed but at 10^{-4} M. No changes were detected for the 4-day-old seedlings. Besides, in the last day of the experiment, there was a high increase in protein contents evaluated to 174.7 µg/seed as compared to control 120.7 µg/seed in developing seedlings under BAP treatment (Figure 4 A).

When seeds were germinating in presence of 10^{-5} M of 20E, proteins levels were decreasing concomitantly with time and drastically from the 3rd day to the 4th day, however a slight drop was observed at day 4 compared to control for the concentration of 10^{-4}M. In all experiments, we found lower amounts of proteins with GA_3 treatment at 10^{-4}M and the most lower ones were detected in presence of 20E at concentration of 10^{-5} M; indeed, protein contents decreased by 28.58, 65.4 and 48% in 3, 4 and 5 days-old seedlings respectively (Figure

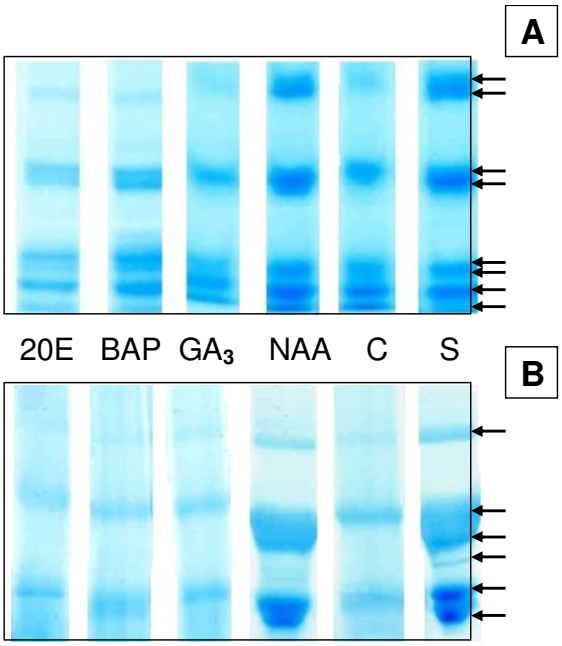

Figure 5. The effect of 10^{-4} M plant hormones and 20-hydroxyecdysone treatment on protein patterns of seedlings from germinating tomato seeds in the 4th day of germination. (**A**) Pattern of supernatant proteins. (**B**) Pattern of protein of the pellet sample. C: Control; NAA: acid naftalene acetic; BAP: benzyl amino purine; GA_3: gibberellic acid; 20E: 20-hydroxyecdysone. S: intact seed (dry seed).

4 A and B).

Effects of treatments on protein patterns

To assess if soluble protein patterns were modified by 20E and hormone treatments, proteins from treated samples were resolved by SDS-PAGE and compared with control. The protein pattern of non-germinated seeds was also given for comparison. Electrophoresis was conducted on days 3, 4 and 5, but we showed only the patterns of day 4 because the protein profiles were similar to the patterns of the other days. The pattern of soluble proteins of the supernatant was given in Figure 5A. We noted that in treated seeds with 20E, BAP, and GA_3 as well as in control the main proteins were mobilized, bands were weakly detected and some of them disappeared during seedling growth in contrast to non germinated seeds. However, NAA inhibited the mobilization and the utilisation of protein and the pattern was identical to that of non-germinated seeds. The same effects of 20E and phytohormones were observed in protein patterns of the pellet (Figure 5B). In NAA treated samples, proteins were detected and were not mobilized during germination and seedling growth in contrast to the patterns displayed in 20E, BAP and GA_3 treated seeds or in control ones.

Effect of treatments on proline content

Proline quantification presented in Figures 6A and B showed that all measurements during the onset of germination and seedlings growth displayed an increase in proline content compared to the one detected within dry seeds. The soluble proline levels in tomato seeds with different treatments were widely different throughout germination and postgerminative growth up to 5 day. In untreated germinating tomato seeds, a very strong increase in proline content took place during emergence and seedling development except a slight decrease in the last day of experiment. Application of NAA showed no effect on proline content at a concentration of 10^{-5} M or a strong decrease in proline content in the 4 days-old seedlings compared to control at 10^{-4} M. In the case of GA_3 treatment, no effect during the four first days then a strong decrease was detected in 5 days-old seedlings compared to control at concentration of 10^{-4} M. In both BAP concentrations used, a decrease in proline content was detected at the 3rd and 5th day, it was much higher in the 4 days-old seedlings. In the case of 20E treatments, the only increase in proline content was detected in the 3rd day of germination at 10^{-5} M, and delayed to day 4 for the concentration of 10^{-4} M.

DISCUSSION

The aim of the present work was to assess changes caused by some treatments of plant hormones such as NAA, BAP, GA_3, and a phytoecdysteroid 20E in germination rate and growth, proline and protein contents. The latter compounds are present in high concentrations in the dry seeds and showed a complex pattern of changes as a result of germination and growth processes. Imbibition is the first step of the process of germination that gives place to the reactivation across processes and metabolic changes that include the degradation of substances of reserves, which remain yet with the emergency of the radicle across the testa (Matilla et al., 2000). Several studies showed that application of several biostimulants and plant growth regulators may increase the germination ability of seeds and seedling vigour in various terrestrial plants (Russo and Berlyn, 1990; Crunkilton et al., 1994; Swaminathaan and Srinivasan, 1996). Matilla in his review (Matilla, 2000) reported that the germination of *chick-pea* seeds depended on ethylene synthesis by the embryonic axis. *Amaranthus retroflexus* seed germination has been shown to be promoted by the plant hormone ethylene (Kepczynski and Kepczynska, 1997). The same behaviour was observed with *Chenopodium album* seeds (Saini et al., 1985a, b), *Lycopersicum esculentum* (Lashbrook et al., 1998) and *Nicotiana tabacum* (Leubner-Metzger et al., 1998).

Among other plant hormones used to improve seed germination of some arboreal species, we can mention

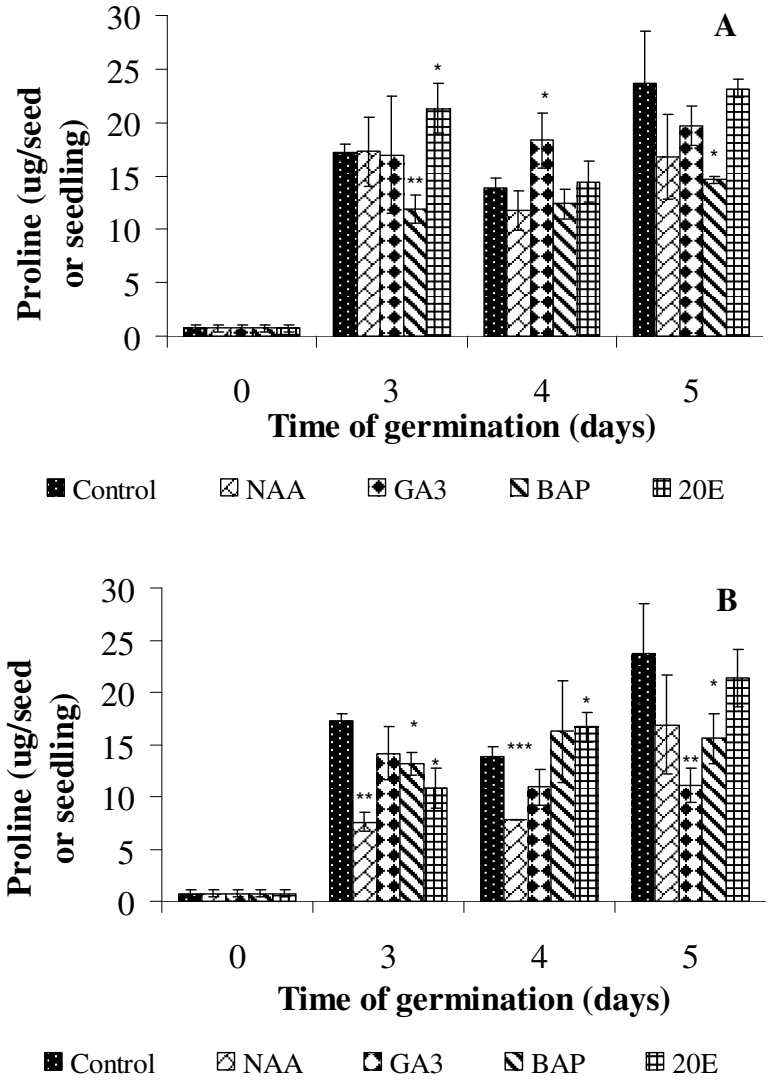

Figure 6. The effect of plant hormones and 20-hydroxyecdysone on proline content in seedlings from germinating tomato seeds. Each value represents the mean + SE of 3 replications. **(A)**: treatment at 10^{-4} M. (**B**): Treatment at 10^{-5} M. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively. NAA: acid naftalen acetic; BAP: benzyl amino purine; GA_3: gibberellic acid ; 20E: 20-hydroxyecdysone.

GA_3, which stimulates germination, cellular elongation and emergency of the radicle across the endosperm and the seed coat (Salisbury and Ross, 2000). These findings support the results of our study concerning germination process. Indeed, all concentrations of GA_3 were effective in promoting germination (Figure 2B). Exogenously added GA_3 had pronounced physiological effects, leading to extended shoot development and germination percentage increase. Groot and Karssen (1987) showed that the germination of tomato seeds depends absolutely on the presence of either endogenous or exogenous GA_3 ; they made clear that the main action of GAs during germination of tomato seed is directed to the weakening of the endosperm cells surrounding the radicle tip. On the other hand, it has been shown that the actions of GA_3, ABA and CKs on lettuce *Lactuca sativa* seed germination are mediated, directly or indirectly, through modulation of protein synthesis (Fountain and Bewley, 1976). Likewise, it has been reported that GA_3, nitrate of potassium and thiourea allows an increase in the germination and viability of the seed of *Peltophorum ferrogeneum* (Mukhopadhayay et al., 1990). A decrease in germination rate was observed under both concentrations of 20E in tomato seeds during the first 3 day of the test period. Similar declines in seed germination have been reported in the literature with some plant hormones. Plant hormone ABA has been shown to inhibit embryonic germination in sorghum (Sharma et al., 2004). Exoge-

nous ABA delayed endosperm rupture and gave rise to a novel structure containing the enlarged radicle with a sheath of elongated endosperm tissue (Leubner-Metzger, 2003). It has been demonstrated that ABA inhibits: (a) phase-III water uptake but does not inhibit phase I or initial embryo-extension growth (Müller et al., 2006); and (b) the endosperm rupture but not seed coat rupture in tobacco and petunia seeds (Kucera et al., 2005).

Differences in shoot and root development were observed in all treatments (Figures 2 and 3). NAA treatments drastically inhibited both shoot and root elongation at any concentration and stage but did not affect germination percentage. Root tissues are sensitive to fluctuating concentrations of auxins (IAA) and the development of the root system can be greatly affected by exogenous sources of this plant growth regulator. At relatively high concentrations, natural auxins, such as IAA, stimulate root induction while reducing root elongation (Tanimoto, 2005). Gravel et al. (2007) showed that exogenous IAA in the rhizosphere can have a detrimental effect on the elongation of roots over a wide range of concentrations. They demonstrated that such an effect has been associated with an increase in the level of ethylene in the plant (Glick et al., 1997, 1998). All concentrations of GA_3 induced high elongation rate compared to other treatments. Literature had already mentioned this statement (Salisbury and Ross, 2000). Benzyl amino purine (BAP) at high concentration and later stages inhibited shoot elongation. However, both concentrations of BAP inhibited strongly root elongation in tomato. Previous work had reported that CKs caused inhibition of the root and hypocotyls in *Arabidopsis thaliana* seedlings and that was coupled to ethylene effects (Cary et al., 1995). 20-hydroxyecdysone caused decline in both root and shoot elongation rate at both concentration used in the last day of experiment. The inhibitory effect may be related to water uptake which could be an important factor in emergence failure as it is for ABA treatment (Demir and Van de Venter, 2000).

In tomato seeds germinated in water, we observed a decrease in proteins content all over the test period. Degradation of storage proteins has already been reported in *Zea mays* during seed germination (Lea and Joy, 1983). Imposition of 10^{-5} M of 20E treatment resulted in a decrease in proteins content during germination and growth processes as compared to others plant hormones whereas GA_3 induced proteins decrease at concentration of 10^{-4} M. In germinating seeds, amino acid are released and transported after hydrolysis of storage proteins (Lea and Joy, 1983) which after catabolism during the early stages of germination provide energy and nutrients for the young metabolically active tissues (Below et al., 2000).

The degradation of storage proteins during seed germination is one of the most important events in the growth and development of seedling. The major amount of protein reserves consists of specific storage proteins like globulins which predominate in dicotyledonous seeds (Shewry and Casey 1999). These proteins are degraded by a variety of proteases into soluble peptides and free amino acids which are mobilized to the embryonic axis to provide energy and support its growth (Shutov and Vaintraub, 1987; Müntz et al., 2001; Schlereth et al., 2001). Phytohormones are involved in up regulation and down regulation of this phenomenon. Our work revealed that NAA inhibited the degradation of proteins during germination and seedling growth in contrast to other tested phytohormones and 20hydroxyecdysone. NAA could act as an inhibitor of protease. Dunavsky and Belozersky (1993) reported that Abscisic acid suppressed the proteolysis of protein by inhibiting, apparently, the synthesis of protease in growing seedling of buckwheat *Fagopyrum esculentum*. On the other hand, exogenously applied phytohormones BAP, AG3 or IAA resulted in stimulation of development of proteases as well as proteolysis in detached cotyledons of Indian bean seeds (Ramakrishna and Ramakrishna, 2005). Decrease in germination rate observed under 20E treatment may be attributed to metabolic alternations. At a concentration of 10^{-5} M of 20E, the decrease in protein contents synchronized with accumulation of proline; this result is in concordance with the data obtained with germinating Indian been seeds showing that accumulation of free amino acids coincided with rapid and maximal proteolysis (Ramakrishna and Ramakrishna, 2005).

On the onset of germination, control and all treatments led to an increase in soluble proline content. This statement has already been mentioned by Lea and Joy, 1983 during seed germination of *Zea mays* as a result of degradation of storage proteins, which generally have high proline content (Lea and Joy, 1983). The increase in soluble proline content may be attributed to the de novo synthesis during germination and growth processes. This was stated by Farrant et al. (1989) and Schwab et al. (1989). They demonstrated that increase in soluble proline is related to de novo synthesis during the first period of rehydration which they compared it to the ones that occurred in salt and drought conditions. It has already been reported that in germinating seeds, in the catabolism pathway of some amino acids, some enzymes such as proline dehydrogenase are regulated during specific phases of the germination process (Nakashima et al., 1998; Goldraij and Polacco, 1999, 2000).

Ecdysteroids represent the steroid hormones of arthropods and probably of many other invertebrates. Phytoecdysteroids are analogues of these invertebrates hormone which occur in a various plant taxa (Dinan 2001). The major phytoecdysteroid present in most plant ecdysteroids-containing plants is 20E, which is also the major ecdysteroid in insects (Lafont et al;, 1991; Lafont 1997). If the effects and application of 20E in animals are extensively studied (Lafont and Dinan, 2003; Dinan and Lafont (2006), its effects and application on plants is rarely studied and the importance of 20E in the life cycle

of plants has not been elucidated. However, there are several evidences that 20E has not a hormonal role within the plants (Lafont 1998; Dinan 2001). Our study related that 20E exogenously applied to seeds of tomato exhibited morphological and biochemical modifications including effects on root and shoot length, a severe reduction on proteins levels owing to the disappearance of some protein bands. This could be explained by a high stimulation of proteins degradation and mobilization by activation of proteases or by an inhibition of proteins synthesis. The sole study conducted on the effects of exogenous application of 20E on high plants showed also stimulatory effects of 20E in wheat *Triticum vulgar*, increase in α-amylase activity in the aleurone layer of barley and enhancement of chlorophyll content in the senescent kidney bean leaves *Phaseolus vulgaris* (Golovatskaya, 2004).

Conclusion

In conclusion, this study showed that 20-hydroxyecdysone fulfils some biological activities in plant physiology. However, if the mode of action of this steroid is well elucidated in animal, particularly in insects. More investigations are needed to understand its mode of action in plants.

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