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Effect of Abscisic Acid on Growth and Certain Osmoregulatory Metabolites in the Leaf Callus of *Gymnocarpos decandrum*

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ABSTRACT. Callus from the desert plant *Gymnocarpos decandrum* was developed and recultured in a medium supplemented with Abscisic acid (ABA). Growth, osmotic potential, water content and some osmoregulatory metabolites were analyzed. Addition of ABA in the callus medium enhanced a callus growth without counteracting other growth regulators in the medium. Added ABA increased callus water uptake, important in resing imposed stresses. The acid also enhanced osmoregulation, regardless of callus water content, by accumulation of some metabolites and not by nutrient elements. The latter were exhausted by increased callus growth activity. Apart of this, there was an increase in the amino acids which reversed ABA inhibitory effect and a decrease in those which enhanced its effect; even some of them could not be detected with time of ABA application. The accumulation of proline was a function of ABA and not of water content, while the reverse was true for quaternary ammonium compounds (QAC).

Direct treatment of plants with sbscisic acid (ABA) has been tried by Mizrahi *et al.* (1974), by spraying seedlings of wheat and barley with (±) ABA and subjecting them to restricted or abundant water supply. Treatment with ABA drastically restricted water loss from the plants, so much so that soil water contents in planted posts were similar to those in pots without plants. In addition, drought-induced senescence of old leaves was inhibited. Also, upon limiting water, ABA treated plants continued to grow whereas the untreated control plants died. Dry matter

Keywords: ABA, callus growth, osmoregulation, proline and QAC.

production by ABA treated plants was greater. Metabolism of ABA in plants was found to occur in the chloroplast in response to stress (Cummins 1973). Loveys (1977) isolated chloroplasts from spinach leaves and found that a considerable proportion (96.6%) of ABA in unstressed leaves was in the chloroplasts, while in stressed leaves only 15.2% of ABA was in the chloroplasts.

Tung and Brady (1972) found application of ABA caused very similar effects to normal senescence as it decreased the incorporation of amino acids into proteins and decreased the proportion of polysomes. Cummins (1973) found that ABA decreased stomatal opening, but the amount of ABA applied to barley leaves was not closely related to the degree of opening of stomata. ABA production plays another role in healthy plants in addition to stomatal control. He found only a minor proportion of ABA can be present in guard cells in the wilted barley leaves. The "extra" ABA present in the mesophyll will stabilize the protoplasm of cells, enabling them to withstand physiological stresses or it could move to and adjust the physiology of other parts. Makeev *et al.* (1992) found that prolonged ABA exposure led to structural reorganization of photosynthetic apparatus and ABA redistribution among photosynthetic products.

Ackerson (1982) found that the ABA level in the exudates of *Gossypium hirsutum* L. leaves was insensitive to the leaf water potential when dehydration occurred over a 3-hour period (*i.e.* ABA accumulated after three hours of zero turgor). Also, slow dehydration of leaves over varying manitol concentration resulted in some accumulation of ABA prior to the point of zero turgor, but ABA accumulated most rapidly after the onset of zero turgor. The same author found stomatal closure prior to accumulation of ABA in the leaf apoplast. Stomata closed at -12 bar and ABA accumulated at -14 bar, with a maximum value at -17.5 bar.

Abscisic acid also occurs in the exudates of leaves, even in the absence of stress-induced synthesis and accumulation arises when leaves are held at zero turgor for several hours (Ackerson 1982). This pre-existing fraction of ABA may represent ABA that is normally synthesized in absence of water stress and is transported to the apoplast for loading into the phloem and its subsequent removal from the leaf (Hoad 1973, Zeevaart 1977).

Drought stress causes rapid accumulation of ABA in many species (Wright and Hiron 1969, Hsiao 1973) and it has been proposed that the hormone may mediate between the imposed stress and the accumulation of organic compounds, particularly proline. Application of ABA led to leaves abscission in unstressed barley plants (Aspinall *et al.* 1973, Rajagopal and Anderson 1978, Stewart 1980).

Desert species are adapted to prolonged water stress during the dry season and facultative water stress between rainfalls during the rainy season. ABA could be mediated in stomatal control or accumulation of metabolites for osmoregulation during stress periods in the desert species *Gymnocarpos decandrum, Plantago albicans* and *Asphodelus microcarpus* (Elhaak and Migahid 1989, Migahid 1995). But exogenous application of ABA at the cellular level in callus of the desert species under normal nutrient and water conditions could elucidate some of the metabolic changes which occur in the desert species with the accumulation of ABA in the plant cells before stress or after stress release and also if these metabolic changes are similar to those during stress.

The aim of the present study was to investigate growth and some metabolic changes that occur in the callus of the desert species *Gymnocarpos decandrum* in response to ABA application.

Materials and Methods

Plants of *Gymnocarpos decandrum,* L. (Caryophyllaceae) were grown from their seeds, collected from the plants natural habitat in the northern part of Egypt, in 15 × 12 cm diameter pots, under greenhouse conditions. The growing plants were continuously irrigated with tap water needed. After the plants became well established (three months old), leaf explants were cultured on MS medium supplied with thiamine, nicotinic acid, pyridoxine, myoinsitol, IAA and kinetin (1, 1, 0.5, 0.5, 100, 3, 0.3 mg/l, respectively), 20 g sucrose as the carbon source and 8 g agar per liter in 250 ml coincal flaks. The cultures were kept at 25 ± 2 °C and 3000 lux with 14 h. photo period, in a culture room under aseptic conditions. The produced calli were removed from the flasks and 0.5 g of calli were recultured in 50 ml of new prepared media, as indicated before. Two groups of prepared callus cultures (each of 18 flasks) were used for this experiment. The first group did not receive any ABA and was considered a control, the second group received 75 μM (2 mg/100 ml culture medium) ABA.

For determination of callus growth, the culture flasks were weighed at different periods of callus growth and compared with the weight of other flasks containing medium only. Two flasks from each group of the callus culture were removed at random at the 0, 2, 4, 6, 8, 10, 14, 18, and 22 days of the experiment. Calli of the two flasks were mixed together freezed under liquid nitrogen, weighed as fresh, lyophylized, and weighed as dry for fresh and dry weights and water content determinations. Dry calli were stored in a deep freezer (–10 °C) for further metabolites analysis.

Dry callus samples (50 mg) were extracted by cold distilled water (for 24 h.), hot distilled water (at 80 - 90 °C for 2 h.), or 70% hot ethanol. Osmotic potentials of distilled water extracts were measured by using an Advanced Wide-Range Osmometer WII, Advanced Instruments, Inc., Germany. Electrical conductivity (EC) of the extract was measured by an electrical conductivity meter (WTW-LF-91, England). Soluble sugars in hot water extracts were measured according to the method of Naguib (1963). Ammonia content was determined by the method of Solorzano (1969), nitrate content by the method of Naguib (1969), and amino acids by the method of Ya and Tunekazu (1966). The constituents amino acids were analyzed by using Beckman amino acid analyzer Model 119 Cl. Proline was measured by the method of Bates *et al.* (1973). Quaternary ammonium compounds (QAC) were extracted and measured according to the method of Stumpf (1984). Cold water soluble proteins were estimated by the method of Bradford (1976). The obtained results were statistically verified by using two way analysis of variance (Snedecor and Cochran 1973).

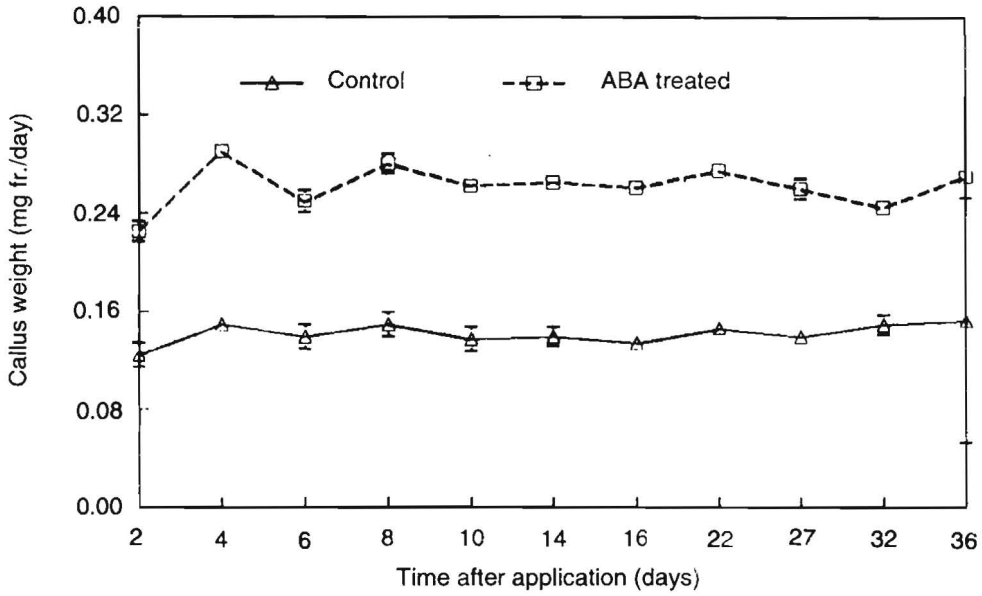

Fig. 1. Fresh weight (mg/day) in control and abscisic acid (ABA) treated *Gymnocarpos decandrum* leaf callus.

Results and Discussion

The study indicated increase in the weight of callus in both control and ABA treated flasks (Fig. 1). The increase in weight was generally greater in ABA treated

callus than the untreated one. More or less similar rates of increase in the callus weight in ABA treated and untreated flasks was obseved in the course of experiment. ABA in well differentiated plants was found to reduce growth through the decrease in CO_2 input via reduction in stomatal opening (Wright 1977). In the callus, as the carbon source was adequate and easily supplied from the medium, growth could be achieved regardless of ABA.

No direct antagonism between the action of ABA and that of both kinetin and gibberellic acid was found by Livne and Vaadia (1965) and Luke and Freeman (1968) . However, exogenous ABA inhibited metabolic activity in the callus of *G. decandrum* when coupled with water stress (Elhaak and Migahid 1989). This suggests that antagonism of ABA to growth hormones could be activated by imposed stress. Exhibition of greater weight by ABA application indicated failure in ABA break down during this long period which is not the state in living plants where ABA fall down faster after stress release. This could be due to the lack of chloroplasts in the callus. The chloroplast was found by Boyer (1976) and Loveys (1977) to be the place where ABA is formed and stored in aerial photosynthetic organs during unstressed periods. This is also supported by the greater rate of destruction of ABA in the mesophyll tissues than in the ground tissue cells which lack such greater number of chloroplasts.

Application of ABA increase water content in the callus as compared to the control (Fig. 2). This indicates enhanced absorption of water from the outside medium in presence of ABA regardless of the water state of cells. Water content of the ABA treated callus increased slightly with time in the course of the experiment. This may be the reason for the increase in callus weight. Water content of control callus decreased slightly by the end of experiment. The water extracts of ABA treated and control callus were acidic; the pH ranged between 5.7 to 5.3. ABA treated callus showed more acidity compared as to control callus throughout the experimental period except at the end (after day 16) when the pH increased slightly in the extracts of ABA treated callus but deceased in that of the control. The decrease in pH was a result of an increase in organic acids rather than in amino acids content (Table 1) which showed negligible or contrasting changes in control and ABA treated callus.

The amount of soluble nutrient elements, as indicated by electrical conductivity (EC) of water extract of ABA treated and control callus, decreased progressively from highest value at the beginning to lowest value at the end of experiment (Fig. 2). This decrease may indicate a lag in nutrients uptake by callus cells or nutrients exhaustion from the surrounding medium. The decrease was more pronounced in ABA treated callus due to its greater growth activity.

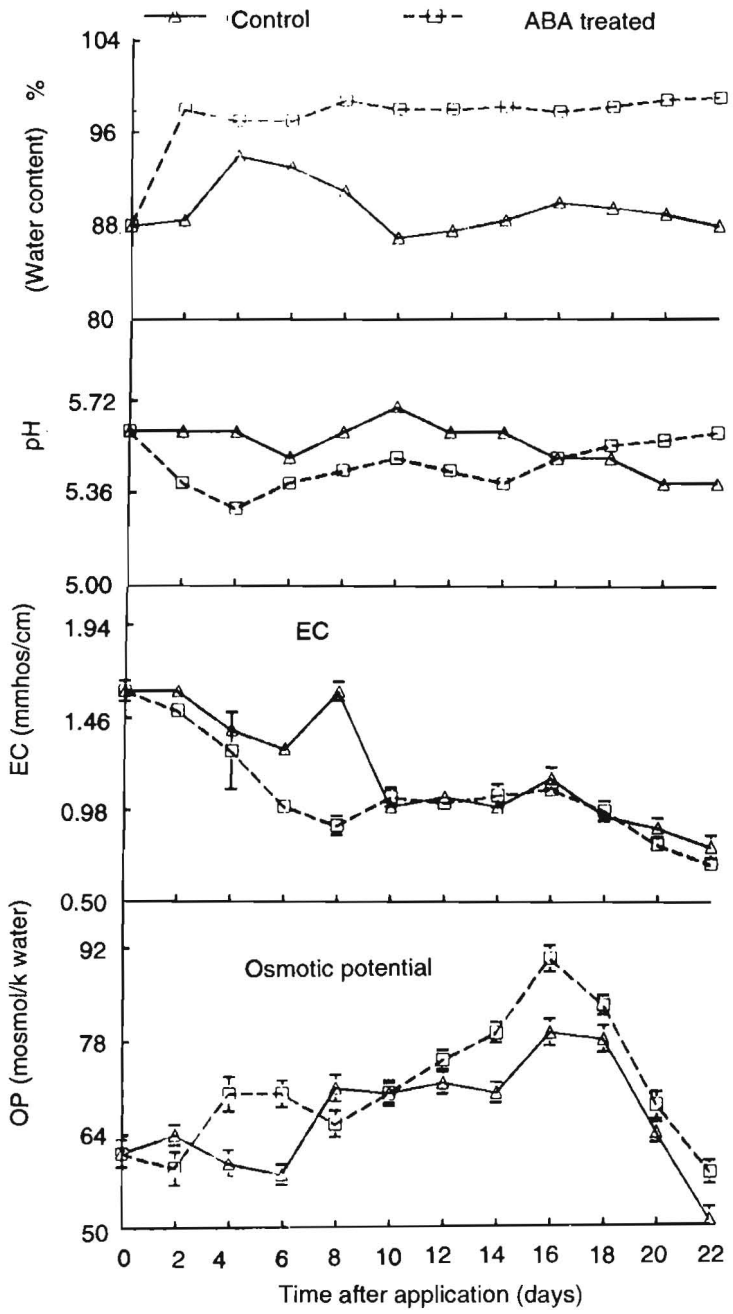

Fig. 2. Water content (%), pH, electrical conductivit (EC) and osmotic potentials (OP) in control and ABA treated *Gymnocarpos decandrum* leaf callus.

Table 1. Amino acids content (mg/100g d. wt) in control and abscisic acid (ABA) treated *Gymnocarpos decandrum* leaf callus.

Recorded amino acids	Control No ABA	Time after ABA application				
		2 days	6 days	10 days	14 days	18 days
Aspartic	1600	1606	1434	617	1187	1624
Threonine	778	774	698	596	611	774
Serine	762	768	675	645	564	768
Glutamic	3430	3433	3761	3784	3639	3323
Proline	1159	1151	768	745	733	921
Glycine	770	771	732	667	619	807
Alanine	1050	1061	1082	903	820	1109
Cystine	7.30	7.97	35.81	15.95	0.00	0.00
Valine	1030	1039	1037	848	784	1089
Methionine	300	298	233	249	236	288
Isoleucine	850	846	736	673	602	877
Leucine	1240	1235	1106	954	937	1176
Tyrocine	580	582	641	453	439	602
Phenylalanine	720	729	847	524	523	787
Histidine	410	409	384	327	278	409
Lysine	1078	1071	994	729	710	1058
Arginine	1940	1937	1850	1689	1518	1781

Differances between the ABA treated and the control in osmotic potential at any sampling date were not significant. But these differences, which were averaged over time, were highly significant ($P < 0.01$). OP of both treated and control callus increased progressively until the 14 to 18 day period after which it decreased noticeably (Fig.2). The increase in OP was notable in the ABA treated than control callus and it was a result of accumulation of metabolites, not of nutrient elements.

Analysis of the callus soluble sugars (Fig. 3) revealed a decrease in soluble sugars by ABA treatment during the experimental period. The decrease was sharp during the first two days of the experiment when the soluble sugars content in ABA treated callus was about half the content of the control. This could be attributed to the high growth activity in ABA treated callus. Soluble sugars content in control callus exhibited high values during the 10 to 16 day period, and decreased to its minimum at the end of the experiment.

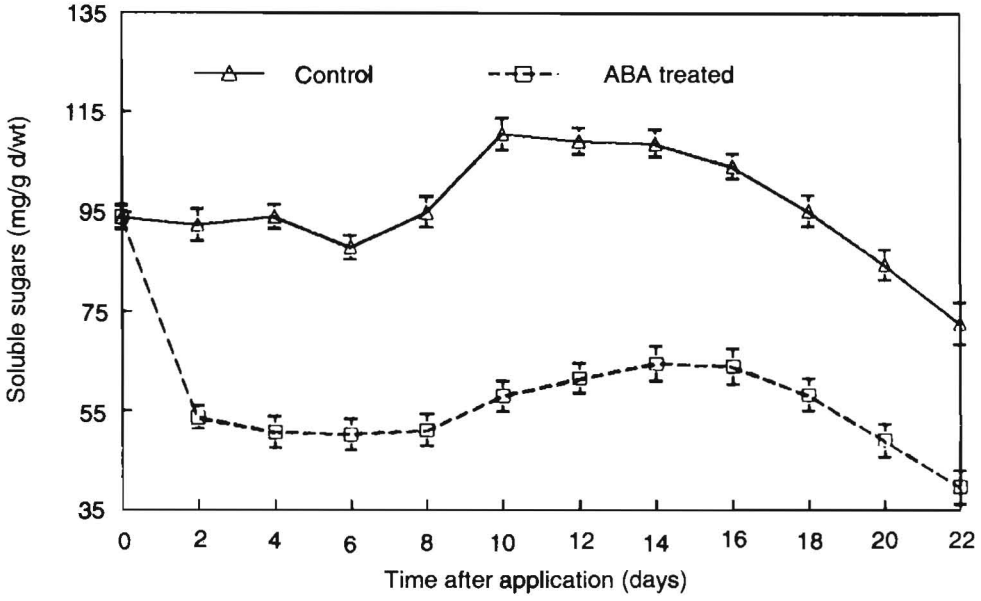

Fig. 3. Soluble sugars content (mg/g d wt) in control and ABA treated *Gymnocarpos decandrum* leaf callus.

The metabolism of nitrogenous compounds in the callus tissue varied greatly with ABA treatment. Ammonia (Fig. 4) decreased slightly in the control callus, whereas it increased in ABA treated callus after a decrease in the first four days. After that, a two to three folds increase in ammonia was recorded with a maximum at the 14th day of experiment. This accumulation of ammonia could be due to an enhancing effect of ABA for nitrogen absorption from the medium. It could also the reason for activation in metabolism of the other nitrogenous compounds.

Hanway and Englehorn (1958) proved that nitrate accumulation in plants is due to several factors, among which drought was possibly the most important. Nitrate increased in the callus by ABA treatment with a maximum value at the eighth day

from application. This increase in nitrate content may indicate that ABA inhibits nitrate utilization by inhibiting nitrate reductase (Mattas and Pauli 1965). Nitrate in ABA treated callus from the 2nd day to the 10th day of the experiment was about double that of control callus. Nitrate in control callus did not show any variations by experimental time until the 14th day, when it increased to the double.

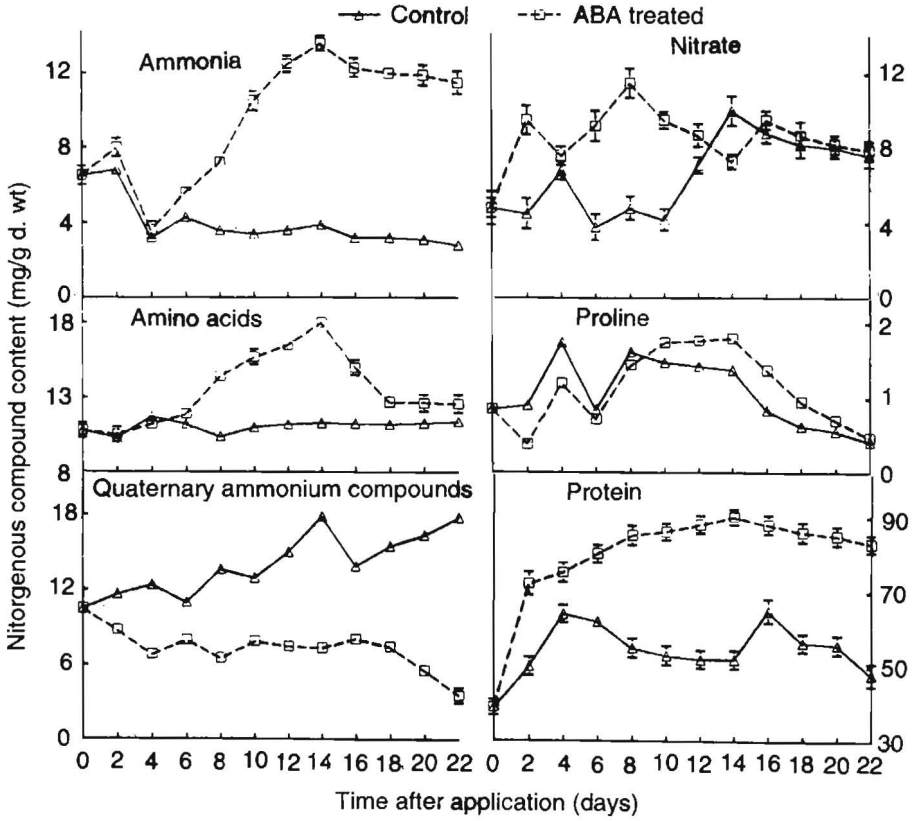

Fig. 4. Ammonia, nitrate, amino acids, proline, quaternary ammonium compounds (QAC) and protein contents (mg/g d wt) in control and ABA treated *Gymnocarpos decandrum* leaf callus.

Amino acids did not show significant variations during the experimental time in control callus (Fig. 4). Application of ABA caused a noticeable increase in the callus content of amino acids during most of the experimental period, with a maximum at the 14th day. Increase in the amino acids with ABA as well as drought stress in *G. decandrum* callus was recorded by Elhaak and Migahid (1989). However, the present study indicates that amino acids accumulated in the callus of the plant in

response to ABA regardless of the callus water content , which was relatively high compared to that of control callus. The constituent amino acids in control callus varied slightly over time , however, the mean value of each amino by time was used as control. ABA treated callus recorded large variations in the content of all amino acids with time (Table 1). The recorded amino acids could be arranged according to the effect of time on their accumulation in the callus into those which decreased with time and those which fluctuated between decreasing and increasing in contents as compared to the control. The first group includes threonine, proline, methionine, leucine, histidine, cysteine and arginine, while the second group includes the rest of recorded amino acids. Application of aspartic acid and glutamic acids was found by Rai and Sharma (1991) to reverse the inhibitory effect of ABA on stomatal opening. However, the increase in the content of both amino acids in the callus after ABA application in the present study could be to counteract the inhibitory effect of ABA. A noticeable decrease was observed in the amino acids which enhanced the effect of ABA, as threonine, proline, leucine, arginine and lysine. Except for the very low per cent with glutamic acid and the high per cent for aspartic acid and cystine, the lowest value of each amino acid were lower than the control value by 22 - 36%. This indicated that about one third of the content of most amino acids was utilized. The results indicate also that glutamic acid exhibited the highest content and cystine the lowest. Aspartic, arginine, leucine, proline, lysine, alanine, and valine respectively came next to glutamic acid. It is also noticeable that the maximum value for most amino acids was recorded during the 14-18 day period of the experiment (Fig. 4).

Proline content increased in ABA treated and control callus (Fig. 4). The increase reached its maximum at the fourth day in the control callus after which it decreased progressively to the minimum value by the end of the experiment. The increase continued in the ABA treated callus until it reached maximum value at the 14th day, followed by a slightly decrease towards the end of the experiment. During the first eight days of the experiment, proline was slightly higher in control callus compared to treated one, but with time treated callus acquired greater content of proline. Similar results of high proline in response to ABA treatment were recorded by Aspinall *et al.* (1973) and (Stewart 1980). In the present study ABA treatment activated proline accumulation in the callus until the 14th day after application compared to the control callus in which proline decreased at the 4th day. Such increase in proline by time of ABA application was also recorded in sunflower leaves by Yurekli *et al.* (1996). The low content of proline in the ABA treated callus, during the first four days, could be a result of an overall inhibition of callus growth during this period.

Quaternary ammonia compounds (QAC) generally increased by experimental time in control callus, whereas the opposite was true for ABA treated callus (Fig. 4).

QAC were recorded to accumulate in *G. decandrum* with drought stress (Elhaak and Migahid 1989). This may indicate that drought stress activated QAC accumulation in the plant and that the presence of high ABA in the cells of the plant does not affect the QAC accumulation process under normal water status of the callus cells.

Protein content increased in both ABA treated and control callus (Fig. 4). The increase was greater in the ABA treated callus throughout the time of the experiment. The maximum value of protein was recorded at the 14th and 16th day of experiment in treated and control callus. After these maxima proteins decreased slightly in both treatments towards the end of experiment. This may indicate that ABA enhanced protein accumulation in the callus regardless of the water status in its cells.

In conclusion, the present study indicates that ABA in the applied dose enhanced callus growth and did not counteract growth regulators in the surrounding medium of the callus. Thus, ABA could be added in the callus culture with other growth regulators and vitamins when there is a need to increase callus resistance to ABA induced stresses such as salinity and drought. Addition of ABA to the medium was found to be important because it enhanced osmoregulation processes, regardless of the water content of callus cells. This was elucidated from its increase to water uptake and osmotic potential in the callus which was reflected on high growth activity in ABA treated compared with control callus. The osmotic regulation was due to metabolic activities rather than the accumulation of nutrient elements which were exhausted in the growth activity. However, there was great accumulation of ammonia, nitrate, amino acids, proline, and protein contents compatible compounds reported (Migahid 1989, Elhaak and Migahid 1989, Migahid and Elhaak 1991) as osmoregulatory compounds in *G. decandrum* under water and salinity stresses. It is important to note that ABA application enhanced accumulation of some amino acids which counteract its inhibitory effects. It also decreased the content of those amino acids which enhanced its effect and even some of them could not be detected by time of ABA application. Accumulation of proline was a function of ABA and not of water content of the callus, while the reverse was true for QAC. This was in agreement with results for *G. decandrum* plants under desert conditions (Migahid 1989).

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تأثير إضافة حامض الأبسيسك على النمو وبعض أيضيات التعديل الأسموزي في كالوس نبات الجرد الصحراوي

محمود أبو اليزيد عبد الحق

قسم النبات - كلية العلوم - جامعة طنطا - طنطا - مصر

تم تنمية كالوس من نبات الجرد الصحراوي ثم أعيدت زراعته في وسط غذائي مزود بحامض الأبسيسك . قيس نمو الكالوس والجهد الأسموزي والمحتوى المائي وبعض المركبات الأيضية التي تدخل في عملية التعديل الأسموزي . وقد اقترح بناء على النتائج التي حصل عليها إضافة حامض الأبسيسك بالجرعة المستخدمة إلى الوسط الغذائي للكالوس لأن هذه الجرعة من الحامض تستحث نمو الكالوس دون ما تأثير على منظمات نمو الكالوس الأخرى في الوسط الغذائي . زيادة على ذلك أدت إضافة حامض الأبسيسك إلى زيادة قدرة الكالوس على امتصاص الماء . وتعتبر هذه القدرة مهمة لمواجهة الجهود التي يتعرض لها الكالوس . كما أدت إضافة حامض الأبسيسك إلى تحفيز عملية التعديل الأسموزي في نسيج الكالوس بغض النظر عن محتواه المائي ، ونتج هذا التعديل بتراكم بعض المركبات الأيضية وليس عن طريق تراكم العناصر الغذائية التي استهلكت من الوسط الغذائي نتيجة لزيادة نشاط نمو الكالوس . كما وجد بأن هناك زيادة في محتوى الأحماض الأمينية التي تعيق التأثير المثبط لحامض الأبسيسك ونقص في تلك التي تحفز هذا التأثير المثبط للحامض حتى أن بعضها لم يمكن رصد تواجده بمرور الزمن بعد المعاملة

بالحامض . ويعود تراكم البرولين إلى معاملة الكالوس بحامض الأبسيسك وليس إلى محتواه المائي بينما كان العكس صحيحا بالنسبة لتراكم المركبات رباعية الأمونيا .