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Characteristic Water Status in Dwarf Pea in Relation to Drought Resistance

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Dwarfism in pea (*Pisum sativum* L.) manifests itself more conspicuously in the light, and light–induced epicotyl growth inhibition in relation to water status was studied. NMR spin–lattice relaxation times (T_1)s of epicotyls of no–elongation zone were markedly longer than those of elongation zone of the seedlings of both cultivars, tall type cv. Alaska and dwarf type, cv. Progress No. 9. T_1 of GA_1–dose response in dwarf pea, which grows normally, decreased in elongation zone while T_1s in no–elongation zone increased. The changes in T_1 of light– and dark–grown pea epicotyls were not simply attributable to a change in water content. Water of dwarf control and no–elongation zone of GA_1–treated cv. Progress No. 9 grown under light condition was more sustained during three–hours air drying compared to those of cv. Alaska. Osmotic concentrations in elongation zones were higher than those of no–elongation zone. In addition, there was no relationship between osmotic concentration and cell length. It seems that the epicotyl tissues of dwarf pea phenotype were high T_1 values accompanied by low osmotic values while water uptake was not changed. In conclusion, T_1 of the pea seedlings will be useful for an evaluation of the dwarfism and GA_1 dose–response to drought stress.

INTRODUCTION

Many dwarf varieties of plants are defective in gibberellin (GA) biosynthesis or sensitivity. A dwarf cultivar of pea plant (*Pisum sativum* L.) appeared to be manifest only in red light while its growth resembled that of tall cultivar when it was grown in complete darkness. Red light suppresses the growth of the shoots of pea seedlings via a phytochrome–mediated response (Sponcel, 1986). Large content of A–2α and A–2β in cv. Progress in the steady state under continuous red light, compared with that in cv. Alaska, may explain the dwarfism of cv. Progress (Noguchi and Hashimoto, 1990). They stated that A–2s were responsible for red light–induced growth inhibition in cv. Progress No. 9. GAs are endogenous regulators of plant growth. Therefore, the inhibition of stem elongation of pea seedlings by light irradiation was also associated with a decrease in the level of GA_1. It has been shown that GA regulates elongation, in both light– and dark–grown hypocotyls in GA–deficient and altered GA–response mutants of *Arabidopsis*

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thaliana (L.) Heyhn cells (Cowling and Harberd, 1999).

GA increased total amount of osmotic solutes in leaf sheath of a dwarf mutant of rice (*Oryza sativa* L. cv. Tan-ginbozu) but did not increase the osmotic concentration of the cell sap, due to an accompanying increase in cell volume by water absorption (Matsukura *et al.*, 1998). Growth of plants is attributed to cell elongation driven by water absorption by cell and cell wall extension (Lockhart, 1965a, b). Water absorption to the cell is governed by the growth-induced water potential (Molz and Boyer, 1978; Nonami and Boyer, 1987; 1993; Nonami *et al.*, 1997; Ikeda *et al.*, 1999a, b). In parenchyma cells, the volume of water occupies 90% or over. Since many metabolic processes occur in aqueous solution in cells, the water in living tissues is considered to play an important role in their physiological condition. Nuclear magnetic resonance (NMR) can be demonstrated to detect the dynamic states of water. Spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) indicate the water status in tissues since they reflect the motion of water molecules. In previous reports, relationship between water content and NMR relaxation times (T_1 or T_2) of water protons has been observed in cold-acclimated red osier dogwood stems (Burke et al., 1974), azalea flower buds (Kaku *et al.*,1984), winter wheat (Yoshida *et al.*, 1997) and wilting flowers (Iwaya-Inoue and Nonami, 2003). Furthermore, the NMR has been applied to the studies of plant tissues that are exposed to environmental stresses (Iwaya-Inoue *et al.*, 1993; Ishida *et al.*, 2000). However, there have been few reports about the role of water in relation to the dwarfism. In addition, it is stated that expression of turgor-responsive gene varies with respect to a time course of induction and reversibility of the wilting-induced changes in shoots of light-grown pea cv. Progress No. 9 (Guerrero *et al.*, 1990).

This paper presents changes in T_1 in epicotyls of tall and dwarf cultivars of pea and GA_1-dose response under dehydration stress. Factors influencing on water status of dwarf pea were also discussed.

MATERIALS AND METHODS

Plant materials

Tall type of pea (*Pisum sativum* L.) cv. Alaska obtaining from commercial sources was used. Furthermore, seeds of dwarf genotype, cv. Progress No. 9, were provided by Dr. M. Katsumi. Two cultivars of pea were cultivated in a growth cabinet (Koitotron HNB-10A) at about 22°C with dark condition or with a photoperiod of 14 h light/10 h dark. Fluorescent lamps (FLR20S W/M 20W×4) were used as a luminous source. 10 to 12 day-old seedlings were used as materials. 100 μM GA_1 was sprayed on an apex of 5 day-old seedlings of cv. Progress No. 9 grown under light condition. Epicotyls of the 2nd node from the apex part as elongation zone or basal part as no-elongation zone were cut from the intact pea seedlings and they were used following experiments.

Measurements of proton T_1 relaxation times

Epicotyl tissues (each about 20 mm in length) were packed into a 7.5 mm diameter NMR tube. NMR measurements were made at 20 MHz on a Bruker Minispec PC20 pulsed NMR spectrometer as described previously (Iwaya-Inoue *et al.*, 1993). The T_1 values of epicotyls were measured by inversion recovery method using a $180°-\tau-90°$ pulse

sequence (Farrar and Becker, 1971). T_1 determination was done 10 to 15 times for each sample. The probe temperature was controlled by a thermostat (Lauda Kryo–SK65) connected to the sample chamber of the spectrometer. Determination was carried out at 20 °C.

Air–drying treatment and measurement of water content of epicotyls

Changes in water content and T_1 were measured by air–drying piece of epicotyls for 30 min to 3 h. Air–drying of section of the tissues was performed by placing the sample on nylon mesh at room temperature (about 26 °C). Fresh and dry weights of epicotyls were obtained after drying in an oven at 80 °C for 24 h. Water content was expressed as the amount of water to dry weight.

Determination of osmotic concentration

Cortex tissues of epicotyls for both cultivars were placed in a series of sucrose solutions. After a treatment period, cells in the sections were examined for plasmolysis under a microscope. The water loss causes a decrease of the protoplast cell volume and eventually leads to plasmolysis. The percentage of plasmolyzed cells in each solution was plotted against the sucrose concentration. The concentration that caused 50% plasmolysis was obtained from the graph and was estimated as osmotic value of individual tissues.

RESULTS AND DISCUSSION

1. Effects of light on cell length and epicotyl growth of pea cultivars

Epicotyl length of pea (*Pisum sativum* L.) dwarf type, cv. Progress No. 9 grown under light condition was about 40 mm while those of tall type, cv. Alaska ranged between 80 and 150 mm (Fig. 1). Under dark condition, the epicotyl of dwarf cv. Progress No. 9 grew well and the length of intact epicotyls was between 130 and 200 mm while cv. Alaska epicotyls grew in a range 260–300 mm. It was indicated that red light inhibited the shoot elongation of cv. Progress No. 9 much more than that of cv. Alaska, and the elongation of cv. Progress No. 9 during 48 h was 23% that of cv. Alaska (Kato–Noguchi, 2002). He described that difference in GA 3β–hydroxylase between cv. Alaska and the dwarf pea was one replacement of alanine with threonine, which may affect the activity of the enzyme. The growth of epicotyl was dependent upon cell length in cvs. Alaska and Progress No. 9 (Fig. 1). Cell length of cortex parenchyma was responsible for the epicotyl growth. GA_1 specifically promoted epicotyl growth of cv. Progress No. 9 grown under light condition (data not shown). A–2α and A–2β may be responsible for the red–light–induced growth inhibition of the dwarf pea seedlings (Kato–Noguchi and Hashimoto, 1997/98). In embryo of *Brassica napus* L. reduced growth of axis treated by uniconazole, GA inhibitor, was due to reduced cell elongation, but not cell number, and the co–applied GA_1 thus prevented the uniconazole–induced reduction in cell length (Hays *et al.*, 2002)

It has been shown that GA regulates elongation, in both light– and dark–grown hypocotyls by influencing the rate and final extent of cellular elongation in GA–deficient and altered GA–response mutants of *Arabidopsis thaliana* (L.) Heyhn cells (Cowling

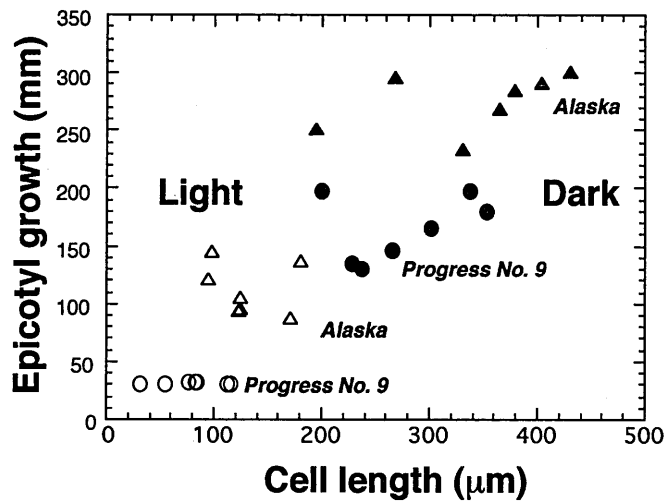

Fig. 1. Relationship between cell length and epicotyl growth of pea (*Pisum sativum* L.) cultivars. Seedlings of Alaska (△, ▲) and Progress No. 9 (○, ●) were grown under light (open symbols) and dark conditions (closed symbols). 10 to 12 day-old seedlings were used.

and Harberd, 1999). However, light- and dark-grown hypocotyls exhibited markedly different GA dose-response relationships. The length of dark-grown hypocotyls was relatively unaffected by exogenous GA, while light-grown hypocotyl length was significantly increased by the GA-dose. Further analysis suggested that GA control of hypocotyl length was close to saturation in dark-grown hypocotyls, but not in light-grown hypocotyls. These results indicated that a large range of possible hypocotyl lengths is achieved via dose-dependent GA-regulated alterations in the degree of elongation of individual hypocotyl cells (Cowling and Harberd, 1999).

2. The relationship between T_1 values and water contents

Since water in pea epicotlys accounts for more than 95% of their fresh weight, NMR signals from non-water protons could be ignored in the following experiment. The relationship between T_1 values and water contents for epicotyls of cvs. Alaska and Progress No. 9 grown at dark condition is shown in Fig. 2A. T_1s for elongation zone ranged between about 0.4 and 0.7 s while those for no-elongation zone were between about 0.9 and 1.3 s in both tall and dwarf cultivars. Epicotyls of no-elongation zone of both cultivars indicated markedly higher T_1 values than those of elongation zone of these genotypes and there was no difference in these cultivars. On the other hand, water contents of both elongation and no-elongation zone were between 17 and 22 g H_2O g dry wt^{-1}. The water content did not relate to T_1 value. Under light condition a marked difference of T_1 between cvs. Alaska and Progress No. 9 was observed in these seedlings (Fig. 2B). T_1 values of cv. Progress No. 9, which mainly consisted of no-elongation zone,

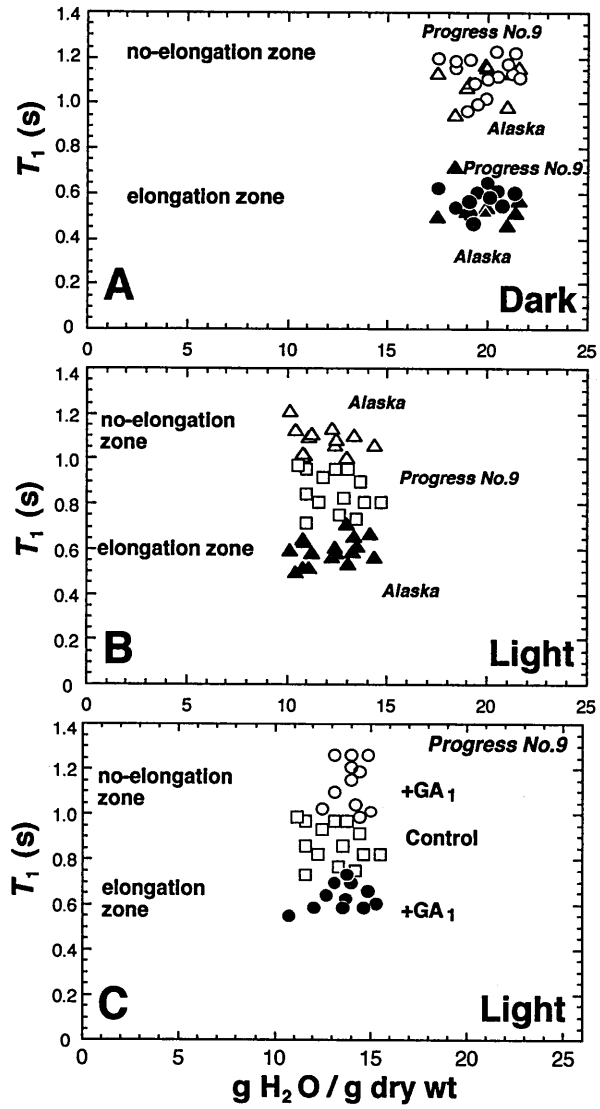

Fig. 2. Relationship between water content and T_1 in epicotyls of pea cultivars. (A) Elongation zone of dark-grown seedlings of cv. Alaska (▲) and cv. Progress No. 9 (●); no-elongation zone of dark-grown seedlings of cv. Alaska (△) and cv. Progress No. 9 (○). (B) Elongation zone of light-grown seedlings of cv. Alaska (▲); no-elongation zone of light-grown seedlings of cv. Alaska (△) and those of cv. Progress No. 9 (□). (C) Effect of GA_1 on light-grown seedlings of cv. Progress No. 9. Elongation zone (●) and no-elongation zone (○) of GA_1-treated seedlings and control seedlings without GA_1 (□). 100 μM GA_1 was sprayed on apex of 5 day-old seedlings.

ranged between those of both elongation zone and no-elongation zone of cv. Alaska. On the other hand, water content ranged between 10 and 15 g H_2O g dry wt^{-1} and there was no difference among three groups. Water contents of light-grown epicotyls were 5 g H_2O g dry wt^{-1} less than those of dark-grown seedlings (Fig. 2A). Present results indicated that higher water content was related to epicotyl growth but not to T_1 values.

Additionally, exogenous GA_1 on cv. Progress No. 9 seedlings grown under light condition induced shoot growth, and it caused markedly changes in T_1 (Fig. 2C). Decreased T_1 of GA_1-dose response was observed in elongation zone while increased T_1s were observed in no-elongation zone in the dwarf pea. There was also no clear relationship between T_1 value and water content. From these results, T_1 values and water contents of GA_1-dose response in the dwarf pea were almost same range in those of no-elongation zone and elongation zone of cv. Alaska, respectively (Fig. 2B, C). Although a marked correlation between T_1 and water content was observed in woody plant leaves (Kaku *et al.*, 1990), the present studies suggested that the change in T_1 of light- and dark-grown pea epicotyls was not simply attributable to a change in water content.

3. Influence of dehydration on T_1 in epicotyls

GA_1-dose response on T_1 and water content was studied by air-drying piece of light-grown dwarf pea. T_1 and water content of epicotyls gradually decreased with air-drying in cvs. Alaska and Progress No. 9 (Fig. 3A). The gradient was the smallest in the elongation zone of cv. Alaska and those of GA_1-treated cv. Progress No. 9 seedling exposed to dehydration stress, and it was indicated that relationship between T_1 and water content was almost same. The fact is supported that T_1 decreased linearly with the decrease in water content of air-drying woody plant leaves (Kaku *et al.*, 1992) and *Vigna radiata* hypocotyls (Iwaya-Inoue *et al.*, 1993). Further, water loss was more enhanced in the elongation zone of both cv. Alaska and GA_1-treated cv. Progress No. 9, and no-elongation zone of cv. Alaska (Fig. 3B). On the other hand, water of dwarf control and no-elongation zone of GA_1-treated cv. Progress No. 9, which was cultivated under light condition, was more sustained during three-hours air drying compared to those of cv. Alaska. However, dwarf type of tall fescue (*Festuca arundinaces* Schreb) indicated more drought-sensitive than tall type based on reductions in relative water content and leaf water potential (Huang *et al.*, 1998). In general, cells of well-watered plants maintain a turgor pressure of 0.4 to 0.8 MPa. Increase in ABA in dehydrated pea shoots could only be detected after 0.5 h-dehydration to zero turgor (Guerrero and Mullet, 1988). From these results, dwarf type was drought resistant compared to tall phenotype even when cv. Progress No. 9 was treated by GA_1.

4. Causative factors affecting T_1 prolongation in dwarfism

It was indicated that high water content was related to epicotyl growth but not to T_1 values (Figs. 1 and 2). Thus, other factors besides water content seem to lead to the prolongation of relaxation times in the dwarfism. Water absorption to the cell is governed by the growth-induced water potential (difference of water potential between the water source and the elongating cells) (Molz and Boyer, 1978; Nonami and Boyer, 1987; 1993; Nonami *et al.*, 1997; Ikeda *et al.*, 1999a, b). Osmotic concentrations in elongation zone were higher than those in no-elongation zone (Fig. 4). In addition, there was no closed

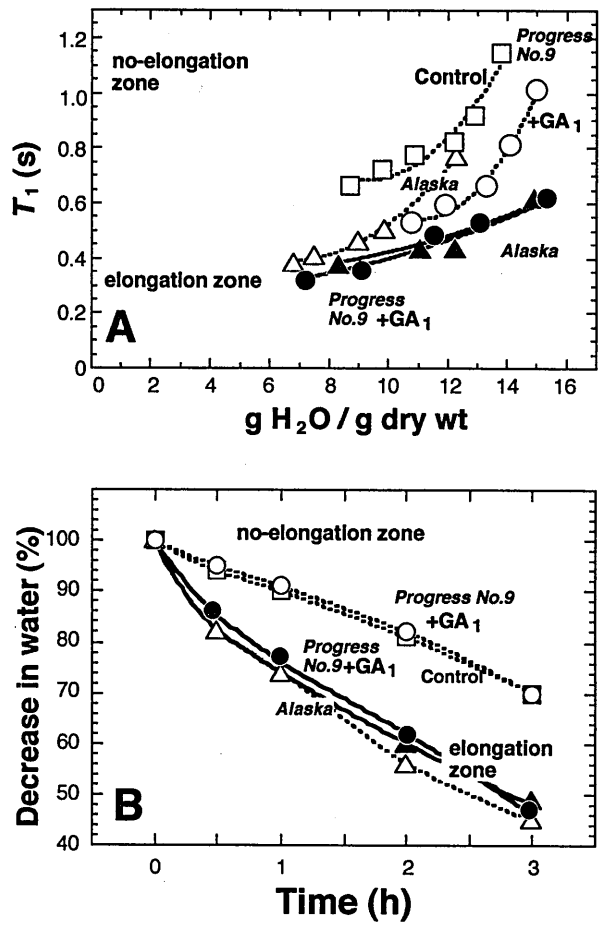

Fig. 3. Influence of air–drying on water content and water status in pea cultivars grown light condition. (A) Relationship between water content and T_1. Elongation zone (▲) and no–elongation zone (△) of cv. Akaska; GA_1–treated elongation zone (●) and GA_1–treated no–elongation zone (○) and control (□) of Progress No. 9. (B) Time course of water loss in epicotyls of pea cultivars. Symbols are Shown in Fig. 3A.

relationship between osmotic concentration and cortex cell length. On the other hand, it has been reported that GA enhanced cell elongation by increasing cell wall extensibility, osmotic concentration being kept unchanged by starch degradation using a dwarf mutant of rice (*Oryza sativa* L. cv. Tan–ginbozu) (Matsukura *et al.*, 1998). Dwarf type of sunflower (*Helianthus annuus* L.) cultivars had up to about 0.4 MPa lower osmotic potential at full turgor compared with a tall cultivar. That is, dwarf sunflower cultivars

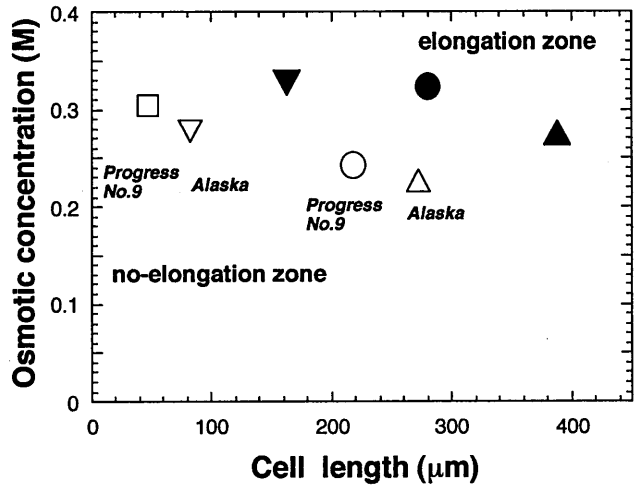

Fig. 4. Relationship between cortex parenchyma cell length and osmotic value of pea cultivars. Open symbols indicate no-elongation zone of cv. Alaska grown under light condition (▽) and dark condition (△), no-elongation zone of cv. Progress No. 9 grown under light condition (□) and dark condition (○); Closed symbols indicate elongation zone of cv. Alaska grown under light condition (▼) and dark condition (▲), and elongation zone of cv. Progress No. 9 grown under dark condition (●).

have ability of greater osmotic adjustment per unit decrease in leaf water potential than the tall cultivar (Angadi and Entz, 2002). Our results suggested that since water contents of both elongation and no-elongation zone were in the same range (Fig. 2), turgor pressure in no-elongation zone might be smaller than those in elongation zone. Generally, T_1s and water content of azalea florets gradually decreased during cold acclimation (Kaku *et al.*, 1984). Further, cold-acclimated azalea petal tissues had high osmotic concentration and thus it seems that the azalea species have a native adaptability as a high mountain species to a cold environment (Kaku *et al.*, 1982). T_1 and T_2 of the sugar solutions linearly depended on their concentrations (Iwaya-Inoue *et al.*, 2002). Therefore, low osmotic concentration in no-elongation zone would partly cause prolongation of T_1 value in dwarf pea.

In conclusion, water status determined by NMR relaxation times (T_1s) in seedling will be useful for a comparative evaluation of the dwarfism and GA dose-response to drought stress. The method revealed that the epicotyl tissues of dwarf type of pea have high T_1 values accompanied by low osmotic values while water uptake was not changed.

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