STRESS METABOLISM

V.* ABSCISIC ACID AND NITROGEN METABOLISM IN BARLEY AND *LOLIUM TEMULENTUM* L.

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Abstract

Spraying a solution of abscisic acid (ABA) of 0·5 or 5·0 μg/ml onto intact barley plants led to an accumulation of free proline in the leaves in certain experiments, as did suspension of the root system in an ABA solution. Proline did not accumulate in the roots of intact barley so treated even when the hormone was taken into the plant through the roots.

Sections excised from the leaves of barley or *L. temulentum* seedlings also accumulated free proline when incubated on ABA solutions. The accumulation resembled the increase in proline concentration produced by incubating the leaf sections on solutions of decreased osmotic potential (polyethylene glycol). With barley leaf sections, incubation on water led to an accumulation of proline with a peak concentration at 48 hr, followed by a rapid loss of proline. Treatment with ABA resulted in an earlier attainment of the peak concentration of proline and a higher maximum concentration. Very little proline leaked out of the tissue into the ambient solution within 4 days of incubation. Excised root segments of either species did not accumulate proline when incubated with ABA.

The data are discussed in relation to the reported increase in ABA concentration in plant tissues subjected to water stress.

I. INTRODUCTION

It has been demonstrated that wilting induces a marked and rapid increase in the concentration of the plant growth hormone (±)*cis-trans* abscisic acid (ABA) in excised wheat leaves (Wright 1969; Wright and Hiron 1969) and intact plants (Mizrahi *et al.* 1970). This accumulation of a potent growth inhibitor during water stress has been associated with various physiological effects of water stress. Rapid stomatal closure in response to exogenous ABA application has been demonstrated (Mittelheuser and van Steveninck 1969; Jones and Mansfield 1970; Cummins *et al.* 1971) and ABA has been suggested as the mediating link between water stress and stomatal aperture control (Cummins *et al.* 1971). This apparent relationship between water stress, ABA concentration, and one physiological response to water stress suggests that an exploration of other physiological and metabolic responses to ABA may be rewarding as an aid to interpreting the effects of water stress.

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Although the mechanism of action of ABA is unknown, it has been demonstrated that RNA synthesis is inhibited by ABA in certain systems (Chrispeels and Varner 1967; Paranjothy and Wareing 1971) leading to a reduction in protein synthesis. Protein synthesis is also inhibited by lowered water potential in plant tissues (Barnett and Naylor 1966) and major changes in free amino acid concentration accompany this response. In particular, plants at reduced water potential accumulate extremely high concentrations of free proline in the leaves and other organs. The potentiality for proline accumulation differs between different tissues of the plant (Singh *et al.* 1973*c*) and is strongly influenced by previous exposure to water stress and by genotype (Singh *et al.* 1973*d*). Treatment with the growth retardant CCC and with gibberellic acid also modifies the extent of proline accumulation (Singh *et al.* 1973*a*). Proline accumulates in excised leaves subjected to water stress (Singh *et al.* 1973*b*) as does ABA, but does not accumulate in excised roots under similar circumstances.

The accumulation of both proline and ABA following induction of stress is a rapid process and it is of interest to speculate whether the phenomena are separate manifestations of the metabolic response of the tissue to the stress or whether, for instance, proline accumulation is a consequence of increased ABA concentration in the tissue. The present paper explores the relationship between applied ABA and proline accumulation in both intact plants and excised tissues.

II. Materials and Methods

(*a*) *Intact Plants*

The effect of ABA on proline accumulation in intact, non-stressed barley plants (cv. Prior) was examined. Seedlings were grown in a constant environment (16 hr photoperiod, 20°C) in perlite irrigated daily with half-strength Hoagland's nutrient solution for 10–14 days, depending on the experiment. At this time some plants were subjected to water stress by flooding the pot with polyethylene glycol solution (mol. wt. 2000, osmotic potential −10 bars); others were sprayed to run-off with solutions containing 0, 0·5, or 5·0 μg ABA per millilitre. Samples of the first leaves and entire root systems were taken 0, 4, 16, and 32 hr after the treatments were applied, and at each time three replicate samples were taken from each treatment and stored for proline assay.

Alternative methods of introducing exogenous ABA into the intact plant were also explored, including entry through a cut leaf and uptake through the roots. Plants exposed to ABA in the rooting medium were grown for 10 days as above and then washed carefully from the sand rooting medium with distilled water. The roots were placed in solutions (15 ml) of ABA of 0, 0·5, 5·0, 50, and 100 μg/ml contained in glass vials, and 24 hr later four replicate plants from each treatment were subdivided into first leaf and remainder of shoot and root systems and then stored for proline assay.

(*b*) *Excised Tissues*

In experiments performed with barley tissues seedlings (cv. Prior) 10–14 days old and grown as before were used. *Lolium temulentum* (single-cycle strain obtained from Dr. L. T. Evans, Canberra) was grown in similar circumstances for 21 days before use. The first leaves of both species were excised and cut into 1-cm sections which were pooled on water before distribution to the various treatments. Root systems of barley and of *L. temulentum* were thoroughly washed to free them from adhering perlite. Whole seedling root systems (barley) or 2-cm tip sections (*L. temulentum*) were assembled on distilled water as they were cut.

A variety of incubation techniques was examined, including incubation in aerated solutions, on moistened filter papers, and on small volumes of solution in Petri dishes. Essentially similar responses were obtained with barley leaf sections in all three situations, although incubation in large volumes of solution without aeration inhibited proline accumulation. In each case, the seg-

ments were incubated at 20°C in the dark. ABA solutions were made up in distilled water immediately before each experiment and stored in the dark; osmotic stress was induced using polyethylene glycol (mol. wt. 2000) solutions. Each treatment was replicated fourfold.

Leaf and root sections were sampled at intervals; 25 disks or an equivalent amount of root tissue per sample was plunged into liquid nitrogen and stored at −20°C before proline assay using a method modified from that of Troll and Lindsley (1955) and Singh *et al.* (1972*c*).

III. Results

(*a*) *Intact Plants*

The effects of spraying ABA solutions onto intact barley plants were variable. In some experiments there was no discernible effect on the concentration of free proline in the plants, whereas in others there was a distinct, concentration-dependent accumulation of proline in the leaf tissues (Fig. 1). The differences in conditions

Fig. 1

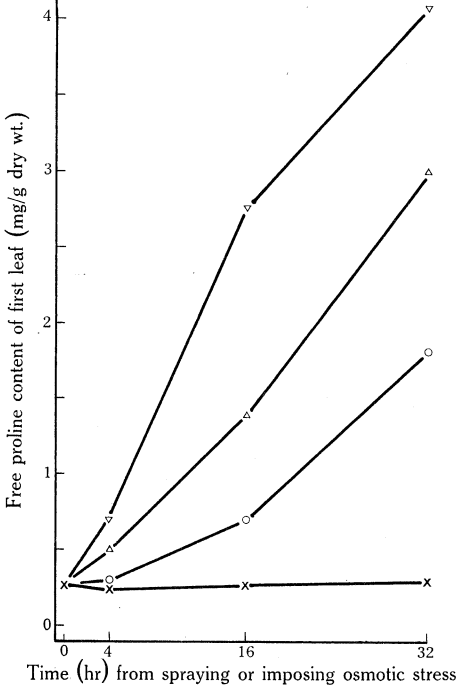


Fig. 2

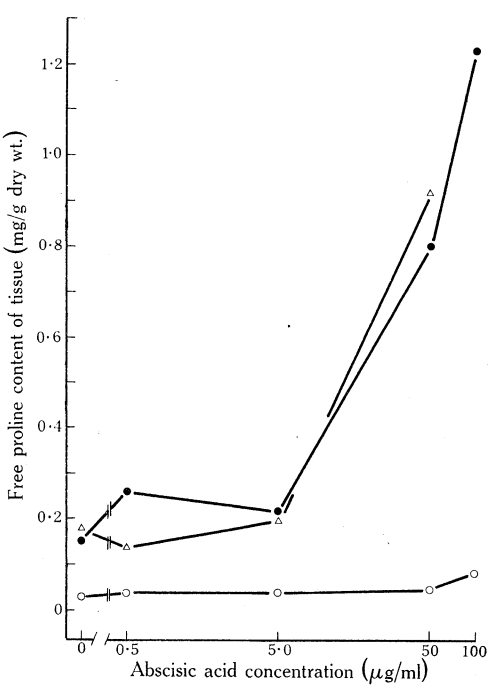
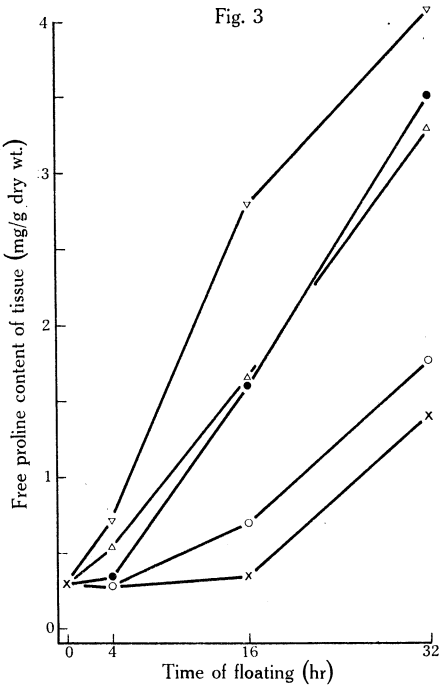


Fig. 1.—Effect of spraying abscisic acid or imposing an osmotic stress on the accumulation of proline in the first leaves of barley seedlings. See text for discussion of variability of results. × Sprayed with water. ○ Subjected to an osmotic potential of −10 bars in the rooting medium using polyethylene glycol. △ Sprayed with 0·5 μg/ml ABA solution. ▽ Sprayed with 5·0 μg/ml ABA solution.

Fig. 2.—Effect of immersing the roots of barley seedlings in abscisic acid solutions for 24 hr on the proline content of the organs of the plant. ○ Roots. ● First leaf lamina. △ Rest of shoot.

between experiments were not easily interpreted, but it was concluded that the variability in plant response was probably due to differences in uptake of ABA into

the plant between the individual experiments. With this in mind, other methods of applying ABA to the plants were explored. Insertion of a cut leaf lamina into a solution of ABA was without effect on proline accumulation, probably due to the small amounts of material absorbed, but uptake through the intact root system produced a consistent increase in free proline concentration in the plant (Fig. 2). There was no ABA-mediated increase in free proline concentration in the plant at a concentration of ABA of 5 μg/ml or less, but a considerable increase in the proline content of the first leaf and the rest of the shoot at an ABA concentration of 50 μg/ml and higher. Although ABA was absorbed through the root system in this experiment, it is noteworthy that there was a negligible accumulation of free proline in the root tissues, even upon exposure to ABA at a concentration of 100 μg/ml. These data are consistent with those of the positive response recorded on spray application of ABA (Fig. 1) and support the supposition that negative responses to applied ABA were due to lack of uptake of the applied ABA by the plant.



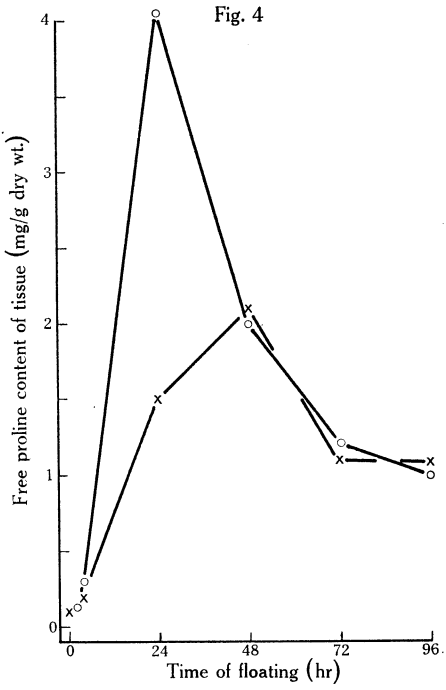


Fig. 3.—Accumulation of proline in excised segments of barley first leaf laminae floating on abscisic acid solutions or polyethylene glycol at 20°C in the dark. × Water. ○ Polyethylene glycol, −10 bars osmotic potential. ● Polyethylene glycol, −20 bars. △ ABA 0·5 μg/ml. ▽ ABA 5·0 μg/ml.

Fig. 4.—Change in the proline content of excised first leaf laminae sections floating for extended periods on water (×) or abscisic acid solution (5 μg/ml, ○).

(*b*) *Excised Organs*

Barley leaf sections accumulated considerable amounts of free proline when incubated on aerated polyethylene glycol solutions of −20 bars osmotic potential (Fig. 3). However, the amounts accumulated by disks on −10 bars solutions were

only slightly more than those accumulated by disks incubated on water alone. As in previous experiments (Singh *et al.* 1973*b*) there was a lag of some 16 hr before disks incubated on water commenced to accumulate proline, but those exposed to −20 bars osmotic potential accumulated appreciable amounts of proline within the first 4 hr. Incubation with ABA caused rapid and extensive accumulation of proline, the rate with 5 μg/ml ABA being higher than that on polyethylene glycol solution at −20 bars osmotic potential.

Very similar results were obtained with *L. temulentum* leaf sections incubated for 24 hr on 5 ml ABA (5 or 50 μg/ml) or polyethylene glycol solutions (−5 or −15 bars), but here the accumulation in response to −15 bars osmotic potential polyethylene glycol solution was greater than that of 5 μg/ml ABA (Table 1).

TABLE 1

ACCUMULATION OF FREE PROLINE IN LEAF SECTIONS OF *L. TEMULENTUM* INCUBATED ON VARIOUS SOLUTIONS FOR 24 HR AT 20°C IN THE DARK

Data analysed statistically following logarithmic transformation. Mean concentrations designated by the same letter (*a*, *b*, *c*) are not significantly different from each other ($P = 0{\cdot}05$)

Incubation medium	Proline concentration (μg/g dry wt.)
Distilled water	418[a]
Polyethylene glycol	
Osmotic potential −5 bars	274[a]
Osmotic potential −15 bars	3579[b]
Abscisic acid	
5 μg/ml	1840[c]
50 μg/ml	2709[bc]

As barley leaf sections incubated on water alone eventually accumulate proline (Fig. 3) it was of interest to determine whether such leaf segments would accumulate free proline after the initial delay to the same extent as those treated with ABA. Furthermore, the rapid metabolism of proline on re-watering water-stressed plants (Singh *et al.* 1973*b*) and the utilization of proline as a substrate for respiration in maize seedling root tips (Oaks 1966) suggests that metabolic utilization of the accumulated proline during continued incubation is a possibility. In order to explore these possibilities, excised barley leaf sections were incubated either in aerated solutions or on 5 ml of solution in a Petri dish. As essentially similar results were obtained in the two circumstances, only those obtained from leaf sections incubated in Petri dishes will be presented here.

Sections incubated on ABA solution (5 μg/ml) demonstrated a very rapid increase in free proline content up to a maximum of 4 mg/g dry weight at 24 hr but the concentration subsequently fell almost as rapidly to 1 mg/g dry weight in the next 48 hr at which level it remained constant for at least a further 24 hr (Fig. 4). The concentration in leaf sections incubated on water also rose steadily during incubation but did not reach a maximum (of 2 mg/g dry weight) until 48 hr, after which the concentration fell in parallel with that in the ABA-treated sections. This suggests

that exposure to ABA serves to accelerate and accentuate the changes occurring in the tissue upon excision from the plant. The maximum difference between water and ABA-treated leaf segments was displayed, in this case, following incubation for 24 hr.

Part of the reason for the decline in proline concentration in the leaf sections after 24 hr incubation could be a release of the accumulated proline into the surrounding medium. This was examined in an identical experiment to the previous one. Again, ABA caused an earlier and more extensive increase in barley in internal free proline concentration followed by a rapid decline in concentration. Only traces of free proline were found in the external solution, however, with leaf sections incubated in aerated solutions or in Petri dishes. There was no evidence for any significant leakage of proline from the sections within the 4 days of the experiment. Evidently the decline in internal concentration is due to metabolism and not leakage.

It has been previously reported that the roots of intact barley plants exposed to an osmotic stress accumulate proline only when adequately aerated (Singh *et al.* 1973*c*). It was suggested that the proline present in the roots of stressed plants was translocated thence from the leaves and this hypothesis was strengthened by the finding that excised, osmotically stressed root systems did not accumulate proline (Singh *et al.* 1973*a*). In view of the accumulation of proline by excised leaf sections in response to applied ABA, it was of interest to explore the response of excised root systems.

Neither complete barley root systems nor apical segments of *L. temulentum* roots accumulated free proline in response to ABA or osmotic stress (Table 2).

TABLE 2

ACCUMULATION OF FREE PROLINE IN ROOT SEGMENTS OF *L. TEMULENTUM* AND BARLEY INCUBATED ON VARIOUS SOLUTIONS FOR 24 HR AT 20°C IN THE DARK

Incubation medium	Proline concentration (μg/g dry wt.)	
	*L. temulentum**	Barley*
Original tissue	3	81
Polyethylene glycol		
−5 bars	0	—
−10 bars	—	55
−15 bars	0	—
−20 bars	—	81
Abscisic acid		
0·5 μg/ml	—	34
5·0 μg/ml	0	36
50 μg/ml	6	68
Distilled water	0	17

* Differences within species were not significant.

Apparently isolated root systems of these species are incapable of independently accumulating proline.

IV. Discussion

The present series of experiments have demonstrated marked accumulation of free proline in the leaves of barley and *L. temulentum* in response to either osmotic stress or treatment with abscisic acid. This response to osmotic water stress appears to be a general phenomenon in higher plants (Barnett and Naylor 1966; Stewart *et al.* 1966). There is less evidence, however, that ABA-induced accumulation of proline is equally universal.

The positive responses to ABA by incised leaf tissue of these two grass species, closely resemble the responses of those tissues to water stress. The relationship between water stress, ABA response, and free proline remains to be elucidated, however. The simplest hypothesis would be that lowered leaf tissue water potential induces an increased synthesis (or release) of ABA such that the concentration of free ABA in the tissue rises. This increased ABA content would then be responsible for a subsequent increase in concentration of free proline.

There is an increasing volume of evidence that a rise in ABA content is a normal consequence of water stress in higher plants (Wright 1969; Wright and Hiron 1969; Zeevaart 1971), but the exact mechanism of this metabolic response to water stress is unknown. The increase in ABA concentration can be detected soon after the induction of water stress (Wright and Hiron 1969), at least as rapidly as accumulation of proline can be detected. However, the relationship between tissue water potential and accumulation is only known for proline (Singh *et al.* 1973*c*) as arbitrary measurements of tissue water content were made in the work with ABA (Wright and Hiron 1969).

Exogenous application of ABA will hasten senescent changes, as measured by a decline in chlorophyll, in the excised leaves of a number of species (Aspinall *et al.* 1967; El-Antably *et al.* 1967; Beevers 1968) and in the leaves of some intact plants (El-Antably *et al.* 1967). The proline accumulation which occurs in barley leaf segments after incubation on water for 48 hr may also be a manifestation of changes in metabolism associated with excision and consequent senescence. The ABA-induced promotion and hastening of this response (Fig. 3) may be viewed as a concomitant effect of the promotion of senescence by ABA. Indeed, the natural rise in proline concentration in excised leaves may also be due to changes in endogenous ABA since Chin and Beevers (1970) have reported an increase in the concentration of endogenous ABA-like substances following the excision of nasturtium leaves. In one respect, however, the effects of ABA on proline accumulation fail to completely resemble those of water stress. Thus, the application of ABA to intact barley plants does not lead to the accumulation of any proline in the roots, whereas proline accumulates in the roots of water-stressed plants, presumably due to translocation from the leaves (Singh *et al.* 1973*c*).

An alternative hypothesis, linking water stress, ABA accumulation, and proline production would be that the changes in metabolism induced by a fall in tissue water potential lead to both ABA and proline accumulation in the tissue. Although the accumulation of ABA would lead to certain physiological responses, particularly stomatal closure, its effect on the proline accumulation mechanism would be solely to reinforce responses already entrained by the fall in tissue water content. Discrimina-

tion between these two hypotheses will require both further examination of species to determine whether ABA application generally leads to proline accumulation, and a comparison of the time courses of ABA and proline accumulation in water-stressed plants.

The absence of accumulation of proline in the excised roots of barley or *L. temulentum* incubated with ABA and even in roots of intact barley plants through which ABA was absorbed (Fig. 2) suggest that the metabolism of the root tissues differs fundamentally from that of the leaves in this respect. As excised root tissues do not accumulate proline when exposed to water stress either (Singh *et al.* 1973*b*), the proline which accumulates in the roots of intact water-stressed plants must be translocated from the shoot (Singh *et al.* 1973*c*). An alternative hypothesis, that leaf-derived ABA is responsible for proline accumulation in the roots of intact plants, is not supported by the present evidence.

V. Acknowledgments

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