Effects of drought stress on the water relations in *Brassica* species

Good, A. G. and Maclagan, J. L. 1993. **Effects of drought stress on the water relations in *Brassica* species.** Can. J. Plant Sci. **73**: 525–529. The physiological responses of different species of *Brassica* to induced drought stress were studied by analysing the relationships between relative water content, leaf water potential and leaf osmotic potential during the onset of drought stress. These data indicate that while there was a decrease in leaf osmotic potential with the onset of drought stress, this did not result from a net increase in solutes. Therefore, these genotypes of *Brassica* do not appear able to osmoregulate under these drought conditions.

Key words: *Brassica*, drought, osmoregulation, water stress

Good, A. G. et Maclagan, J. L. 1993. **Effet du manque d'eau sur les rapports hydriques dans les espèces du genre *Brassica*.** Can. J. Plant Sci. **73**: 525–529. Les réactions physiologiques de différentes espèces de *Brassica* à un stress hydrique artificiel ont été analysées d'après les rapports obtenus entre la teneur eu eau relative, le potentiel hydrique des feuilles et le potentiel osmotique des feuilles durant la phase de déclenchement du stress. Les observations recueillis montrent que, même si le potentiel osmotique diminue avec l'arrivée du stress, cette réaction n'était pas le résultat d'un accroissement net de la concentration de la solution cellulaire. Il semble donc que les génotypes considérés ne possèdent pas l'aptitude de s'osmoréguler dans des conditions de sécheresse.

Mots clés: *Brassica*, sécheresse, osmorégulation, stress hydrique

With most plants, the maintenance of growth and function depends on maintaining a relatively high water content in the protoplasm. This is because many important physiological processes such as leaf enlargement, stomatal opening, and photosynthesis are directly affected by a reduction in leaf water potential (Hanson and Hitz 1982). In crop plants, water deficits have been shown to decrease both growth and yield (Richards and Thurling 1978a; Morgan 1984). Breeding higher-yielding crops for drought-prone environments has, in the past, been accomplished by selecting directly for yield. An alternative to this empirical approach to the genetic improvement of drought resistance in crop plants is to first identify the relevant drought resistance mechanisms and then develop suitable methodologies for their measurements (Richards and Thurling 1979).

Osmotic adjustment, i.e., a net increase in solutes leading to a lowering of osmotic potential is one of the main mechanisms whereby crops can adapt to limited water availability (Turner 1979; Morgan 1984). The solutes that accumulate during osmotic adjustment include sugars, amino acids, organic acids, proline and glycine betaine (Hanson and Hitz 1982). Corn, cotton, soybean and wheat have all demonstrated osmotic adjustment during drought (Morgan 1984).

In the *Brassica*, it has been shown that there is variation in response to drought stress both between and within the species *B. rapa* and *B. napus*. Richards and Thurling (1978a,b) demonstrated that drought stress markedly influenced seed yield and its components in different cultivars of *B. rapa* and *B. napus*. This paper examines the physiological responses to drought stress of three genotypes representing the species of *Brassica* in terms of the relationship of leaf relative water content with both leaf water potential and leaf osmotic potential. The response to drought stress was measured with a view to comparing the drought tolerance of the different *Brassica* species and their ability to osmoregulate.

Individual seeds were planted 1 cm deep in 13-cm-diameter plastic pots containing a soil and fertilizer mixture as described by Stringam (1971). These pots were placed in growth chambers under the following conditions: (i) 16 h of 265 μmol m^{-2} s^{-1} provided by VITA-LITE U.H.O fluorescent tubes, (ii) day and night temperatures of 21°C and 15°C, respectively, (iii) relative humidity of 85-97%. Upon germination, all seeds were watered regularly and allowed to grow until the first flowers appeared on the plants. From this stage onwards, water was withheld from one half of the plants while the other half of the plants continued to be watered daily; approximately 3 h before measurements were taken. The following measurements were conducted daily at the same time during the day, on four droughted plants or four control plants. Each experiment was repeated independently a minimum of three times.

All measurements of relative water content were made on the second fully expanded leaf on each plant. The leaf and a portion of the petiole were cut from the plant and immediately weighed. They were then placed in water for 2 h until the leaves achieved full turgor. The leaves were removed from the water, blotted dry and reweighed. The leaves were then placed in an 80°C oven for 24 h and weighed to obtain dry weight measurements.

A thermocouple psychrometer (DECAGON SC-10A; DECAGON DEVICES INC. Pullman, WA) connected to a nanovoltmeter/thermometer (DECAGON NT-3) was used to measure leaf water and osmotic potentials as described by Morgan (1983) and Grumet and Hanson (1986). All measurements were made on the first fully expanded leaf of each plant. Water potential was measured on a leaf strip, 11 × 45 mm, cut from midway between the edge and the mid-vein of the leaf. This strip was placed around and in close contact with the inside walls of the sample cup. The cup was placed in the psychrometer and allowed to equilibrate for 30 min. The remainder of the leaf was rolled and inserted into a 20-cm length of TYGON tubing. Clamps were used to seal the ends of the tube before it was frozen in liquid nitrogen. The tubing and tissue were then quickly thawed and pressed between two rubber rollers. Using a Pipetman, 400 μL of solute was drawn directly from the tubing and placed in a sample cup lined with an 11 × 45-mm strip of Whatman no. 1 filter paper. The cup was quickly loaded into the psychrometer and allowed to equilibrate for 30 min. The mV and temperature readings were recorded and converted to water potential and osmotic potential.

The genotypes used were *Brassica napus* (cv. Westar; 37 d to flowering (dtf), *B. rapa* (cv. Eldorado; 27 dtf) and *B. juncea* (cv. Cutlass; 8 dtf). Figure 1A illustrates the decrease in relative water content (RWC) over time for the different cultivars. The decrease in RWC for Westar occurred slowly but continuously. After the 5th day the RWC had decreased to 57% and the leaves were unable to rehydrate. In contrast, the RWC of Cutlass decreased much more rapidly during the first 3 d, decreasing to 37% after 5 d of imposed drought stress. The RWC of Eldorado decreased to the lowest level 30%, before being unable to rehydrate.

The effect of drought stress on the leaf water potential (WP) of the different genotypes is shown in Fig. 1B. All genotypes had WPs that ranged from −0.6 to −1.1 MPa when watered. With the cessation of watering, the water potential of the leaves decreased; however, the rate and degree of decrease varied. Westar decreased to −2.0 MPa after 5 d of drought, whereas Eldorado and Cutlass decreased to −3.0 and −3.4 MPa, respectively. The change in leaf osmotic potential (OP) during drought stress is shown in Fig. 1C. Osmotic potential is presented in two ways. To determine whether the changes in OP

Fig. 1. Average (±SE) relative water content (1A), leaf water potential (1B) and leaf osmotic potential (1C) of leaves of *B. napus* 'Westar', *B. rapa* 'Eldorado' and *B. juncea* 'Cutlass' with days after the onset of drought stress. Open squares are controls, closed squares are droughted plants.

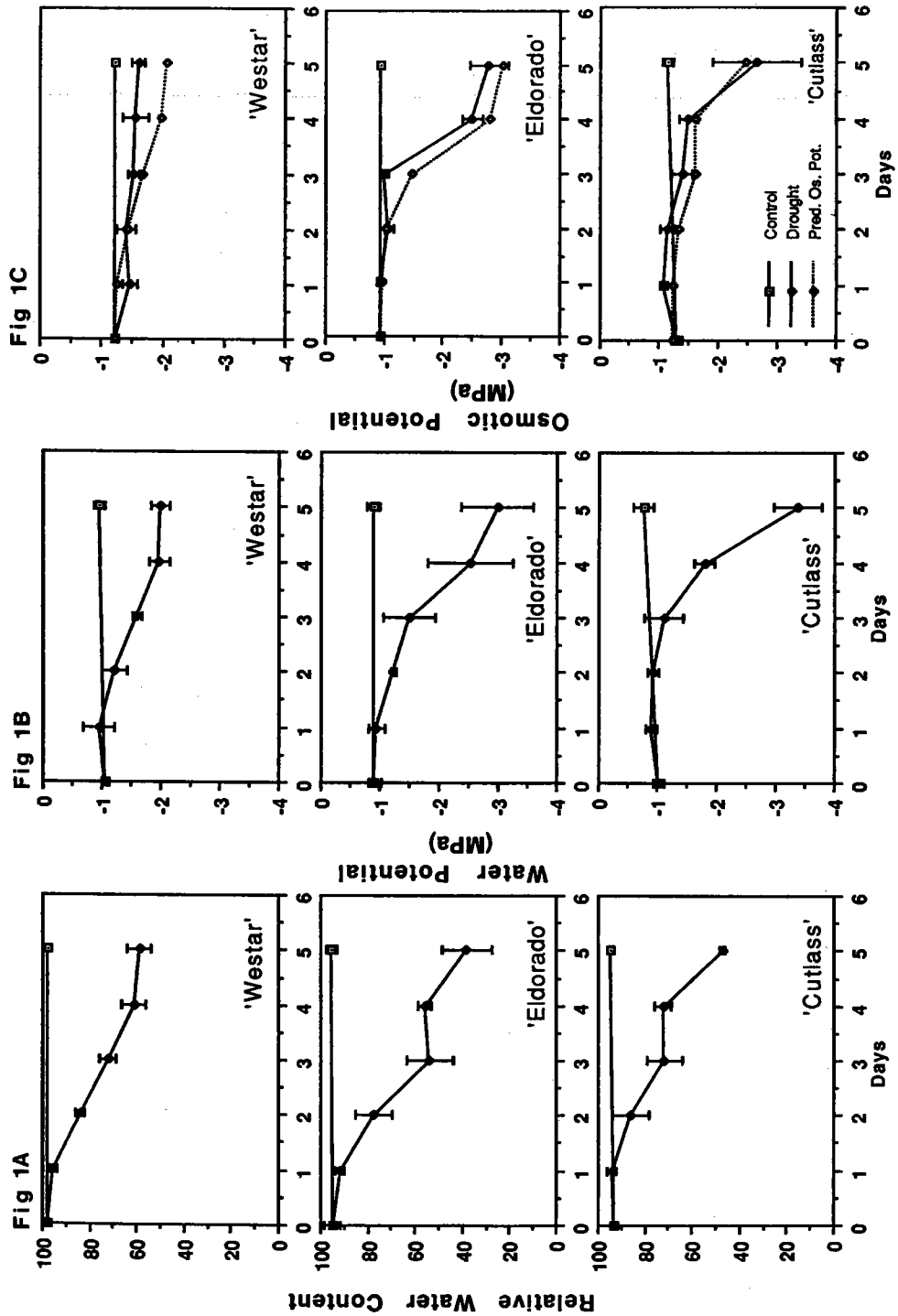
Fig 1A
Relative Water Content
'Westar'
'Eldorado'
'Cutlass'
Days
Fig 1B
Water Potential (MPa)
Fig 1C
Osmotic Potential (MPa)
Control
Drought
Pred. Os. Pot.

were a result of solute accumulation instead of leaf dehydration, predicted OP measurements were calculated by correcting the control OP to 100% as described by Morgan (1984). This equation predicts the change in osmotic potential caused by the concentration of solutes resulting solely from loss of water from the cell. Therefore, when solute accumulation occurs, the OP will be less (more negative) than the predicted OP. For Westar and Cutlass, the leaf osmotic potential stayed relatively constant for the first 2 d and then proceeded to decrease as the RWC of the leaves decreased. However, the decrease in osmotic potential was not sufficient to maintain the turgor of the leaves, as all of the plants showed visible signs of wilting by the third day. The decrease in OP was most noticeable in Eldorado; however, most of this decrease in OP was as a result of dehydration of the leaves. None of the cultivars had measured OPs that were lower than the predicted osmotic potential during the onset of drought stress (Fig. 1C).

This preliminary study outlines the changes in plant water relations in *Brassica* during the onset of induced drought stress. Drought has been shown to have a marked effect on seed yield and its components in a number of different cultivars of *B. napus* and *B. rapa* (Richards and Thurling 1978a,b). We chose to study the effect of drought on water relations during the onset of flowering, because yield is reduced the most in both *B. napus* and *B. rapa* when plants experience drought at this time (Richards and Thurling 1978a). We demonstrated that clear differences exist among the different *Brassica* species in their water relations during imposed drought stress. A comparison between the osmotic potential of leaves before rehydration and the predicted osmotic potential based on full turgor (Fig. 1C) demonstrates that while there was a decrease in leaf osmotic potential with the onset of drought stress, none of this resulted from a net increase in solutes. Moreover, this change in osmotic potential with the rapid decrease in RWC of the leaves during the onset of drought stress is not sufficient to maintain leaf turgor. Therefore, these genotypes of *Brassica* do not appear to be able to osmoregulate under these simulated drought conditions. We feel that this inability to osmoregulate may result primarily from the rapid onset of drought under these laboratory conditions.

There are several ways to view drought tolerance. A number of authors have evaluated drought tolerance based on the number of days the plant was unwatered and yet able to fully rehydrate its leaves. Based on that criterion it would be difficult to determine which genotype was most drought sensitive. Alternatively, one could evaluate drought tolerance based on the ability of a genotype to retain a high leaf relative water content. For example, Westar decreased to a RWC of 59% after 5 d before being unable to rehydrate, whereas Eldorado was able to decrease to a RWC of 38% after 5 d, at which point the leaves were unable to rehydrate (Fig. 1A). However, since neither genotype was able to rehydrate after this, Westar's ability to retain a high leaf RWC has not improved its drought tolerance under these conditions. Variation in drought tolerance between different cultivars or species has often been attributed to differences in their time to maturity (Richards and Thurling 1979). On this basis it would be expected that *B. rapa*, the earliest flowering species, would be better adapted than *B. napus*; however, this does not appear to be the case. One factor that might contribute to the differences in drought tolerance is the pattern of dry matter accumulation and growth during the plant's development. For example, Thurling (1974) found that at least 85% of the total dry weight of *B. rapa* cultivars was accumulated after anthesis, whereas for *B. napus* the dry matter accumulation after anthesis represented only 55% of total dry weight. Thurling (1974) suggested that *B. rapa* may be more susceptible than *B. napus* to drought since it flowers earlier and accumulates more of its dry matter after flowering. We found a similar correlation between the time to flowering and drought tolerance. These initial studies have allowed us to characterize the water relations in *Brassica* species during imposed drought

stress. However, before this approach can be used to rank genotypes in terms of drought tolerance, these results must be compared with results from field studies.

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