**Pod Set Related to Photosynthetic Rate and Endogenous ABA in Soybeans Subjected to Different Water Regimes and Exogenous ABA and BA at Early Reproductive Stages**

FULAI LIU1*, CHRISTIAN R. JENSEN1 and MATHIAS N. ANDERSEN2

1The Royal Veterinary and Agricultural University, Department of Agricultural Sciences, Højbakkegaard Allé 9, DK-2630 Taastrup, Denmark and 2Danish Institute of Agricultural Sciences, Department of Crop Physiology and Soil Science, Research Centre Foulum, PO Box 50, DK-8830 Tjele, Denmark

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*For correspondence. E-mail: fl@kvl.dk

- **Background and Aims** The physiological reasons for reduced pod set in soybean (Glycine max) caused by drought during anthesis are not established. The objective of this study was to investigate the involvement of photosynthetic rate (A), pod endogenous abscisic acid (ABA) and exogenously applied ABA and 6-benzylaminopurine (BA) in regulating pod set in soybean during drought.
- **Methods** Two pot experiments were done in a controlled-environment glasshouse. In expt I, soybeans were either well-watered (WW) or droughted by withholding water from 4 d before to 4 d after anthesis (DAA). In expt II, soybeans were drought-stressed (DS) from −4 to 4 DAA. From −2 to 4 DAA, some of the WW and DS plants were treated with 0.1 mM ABA or 1 mM BA.
- **Key Results** Drought stress decreased A, but increased pod ABA concentration ([ABA]). Pod set decreased only when A had decreased by 40 %, and pod [ABA] had increased 1.5-fold. Beyond the thresholds, pod set correlated positively with A and negatively with pod [ABA]. Exogenously applied ABA decreased A and pod set in WW plants, whilst it increased A and pod set in DS plants; exogenous BA had opposite effects. In these plants, pod set correlated linearly with A.
- **Conclusions** Drought-induced decrease in A is significant in inducing pod abortion, probably as a consequence of carbohydrate deprivation. The effects of ABA and BA on pod set may be partially due to their effects on photosynthetic supply.

**Key words:** Glycine max, abscisic acid, 6-benzylaminopurine, drought stress, photosynthetic rate, pod set.

**INTRODUCTION**

In soybean, reproductive potential may be reduced considerably due to abscission of developing flowers and pods soon after anthesis during pro-embryo development [3–5 d after anthesis (DAA)] (Peterson et al., 1990) even under optimal environmental conditions (Carlson et al., 1987). This stage is one of active cell division in the young ovules, coinciding with a rapid pod expansion (Peterson et al., 1992), which is particularly sensitive to drought stress (Westgate and Peterson, 1993). Although there is no consensus about the physiological mechanisms controlling pod abortion during this critical stage under drought stress, factors that slow cell division and expansion within the ovaries, such as the availability of assimilate and changes in the endogenous concentrations of certain phytohormones, particularly ABA and cytokinins (CKs) are probably involved. Experimental evidence from cereals (e.g. maize) and grain legumes (e.g. lupin) has indicated important roles of these factors in regulating kernel/pod set under drought conditions (Hoad, 1978; Westgate et al., 1996; Palta and Ludwig, 1997; Saini and Westgate, 2000; Setter et al., 2001).

Photosynthetic rate plays an important role in controlling crop reproductive development under well-watered conditions. Manipulation studies involving altered radiation intensities (Egli and Yu, 1991), elevated atmospheric CO2 (Mitchell et al., 1996; Palta and Ludwig, 2000), and supplying sucrose via stem injection (Abdin et al., 1998) have demonstrated that there is a linear relationship between the rate of assimilate supply and the number of fruit/seed per unit area of land surface for several crop species, including soybeans. It is also well known that the export rate of sucrose from source (leaves) to sink (fruits/seeds) organs correlates linearly with the current photosynthetic rate (A) (Huber et al., 1984). Soil water deficits reduce A and thus the amount of photosynthate allocated to floral organs and may, thereby, increase the rate of fruit/seed abortion. In drought-stressed maize (Zea mays), a fundamental role of current photosynthate supply in determining kernel set has been proposed (Schussler and Westgate, 1995; Setter et al., 2001). However, this aspect has not been investigated in soybeans.

Phytohormones may also regulate plant reproductive development in response to drought stress. The ABA concentration ([ABA]) increased significantly in the xylem sap, leaves and reproductive structures of drought-stressed plants (Hoad, 1978; Westgate et al., 1996; Setter et al., 2001; Liu et al., 2003). Soil drying reduced seed set in wheat (Triticum aestivum) (Westgate et al., 1996) and maize (Setter et al., 2001), and pod set in soybean (Liu et al., 2003).
2003) has been ascribed to the elevated [ABA] in reproductive structures. In addition, manipulation studies have shown that exogenous application of ABA to leaves or to ears reduced seed set in maize (Myers et al., 1990) and wheat (Wang et al., 2001) supporting a role of greater [ABA] in reducing seed set in drought-stressed crops. A negative linear relationship between seed set and [ABA] in floral organs has been observed in drought-stressed wheat (Westgate et al., 1996). However, the extent to which changes in endogenous pod [ABA] affect pod set is unknown, or if exogenous ABA induces pod abortion in soybeans.

Apart from ABA, CKs play an important role in regulating crop reproductive development (Hare et al., 1997). Application of cytokinin-like substances, e.g. 6-benzylaminopurine (BA), to individual racemes or to the transpiration stream can prevent pod abortion in well-watered soybean (Carlson et al., 1987; Nagel et al., 2001) and lupin (Lupinus angustifolius) (Atkins and Pigeaire, 1993; Ma et al., 1998). It is suggested that CKs reduce soybean and lupin pod abortion by increasing sink strength and redirecting the movement of assimilates into treated tissues (Reese et al., 1995; Ma et al., 1998). Therefore, it is plausible to suggest that exogenous application of CKs to drought-stressed plants might prevent pod abortion. However, this has not been studied.

In the current study, A and pod [ABA] were quantified in soybeans subjected to different durations, and thus intensities, of drought stress during early reproductive development. The effects of exogenously applied ABA and BA on pod set in both WW and DS soybeans were also investigated. The objective was to establish how drought-induced changes in A and endogenous [ABA], and exogenous applied ABA and BA relate to pod set.

MATERIALS AND METHODS

Plant material and growing conditions

Two pot experiments (expts I and II) were done at the experimental station of the Royal Veterinary and Agricultural University (KVL), Taastrup, Denmark. Soybeans (Glycine max L. Merr. ‘Holladay’, supplied by Dr Tommy Carter, North Carolina State University, USDA, ARS, USA) were sown in pots (15-cm diameter and 25-cm tall) containing 0.5 kg compost (GB-Pindstrup Substrates No.1, pH 6-0). Seeds were inoculated with Bradyrhizobium japonicum (MicroBio Ltd, Cambridge, UK) before sowing. Three seeds were sown and plants thinned to one per pot when the first trifoliate leaf emerged. Plants were grown in a glasshouse where day/night air temperature was 28/22 ± 2°C; relative humidity was 60 %; photoperiod was 13 h with 600 μmol m−2 s−1 (PAR) supplied by metal-halide lamps. Pots were randomized in the glasshouse daily.

Water supply treatment

At the beginning of the experiments, pots were watered and allowed to drain freely to constant mass (about 3.4 kg); this was 100 % water holding capacity (WHC). Two weeks after emergence, all plants were well watered (WW) daily to 95 % WHC with nutrient solution (Pioneer NPK Macro 14-3-23+Mg combined with Pioneer Micro; pH 5.5; EC 1.3) using automatic drip irrigation. Plants were irrigated with tap water without nutrients from −4 DAA. In expt I, irrigation was withheld from eight pots each day from −4 (DS6), −3 (DS5), −2 (DS4), −1 (DS3), 0 (DS2), and 1 (DS1) to 4 DAA. Thereafter half were re–watered to 95 % WHC with nutrient solution until physiological maturity. For the control treatment, eight plants were WW at 95 % WHC throughout the experimental period, but without nutrients from −4 to 4 DAA. In expt II, soybeans were drought stressed (DS) from −4 to 4 DAA, with WW plants as controls. On 4 DAA, half of the plants were harvested and the rest were WW until maturity.

Exogenous application of ABA and BA

In expt II, from −2 to 4 DAA, 0-1 μM (±)-cis, trans-abscisic acid (ABA; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 1 μM 6-benzylaminopurine (BA, a synthetic CK; Sigma-Aldrich Chemie GmbH) were each applied, using a hand-held aerosol propelled sprayer, separately, every day, to the canopy (including leaves and racemes) of eight WW and eight DS plants each until incipient runoff (approx. 10 mL aqueous solution per plant). The control plants (eight WW and eight DS) were sprayed with the same amount of distilled water. ABA and BA were dissolved first, in very small volumes of ethanol, and then with de-ionized water containing 0-1 % Tween 20 as wetting agent (Cho et al., 2002).

Pod sampling

In both experiments, flowers on racemes from main stem nodes (7–11) in the middle of the canopy were tagged before anthesis and their development and growth were monitored. All flowers opened within a period of 2 d. The date of flowering (anthesis) was determined when the banner petal had fully extended in length (B2 stage according to Peterson et al., 1992). In expt I, pods were sampled 4 DAA, immediately weighed, then frozen in liquid nitrogen and stored at −80 °C before analysis. In both experiments, the number of flowers was counted at 0–1 DAA and pods that developed from them counted every 3 d until the rate of pod-set became constant. Pods more than 2 cm long were considered to be fertile. The percentage of pod set was calculated as the number of fertile pods divided by the number of flowers.

Measurements of stomatal conductance (gs), A, and leaf water potential (LWP)

In expt I at sampling (4 DAA) and in expt II from 0 to 4 DAA, gs and A were measured on the second, fully expanded leaf in the upper canopy (one leaf per plant, four plants per treatment). Measurements were made between 1100 and 1300 h at photosynthetic light saturation (PAR > 800 μmol m−2 s−1), sunlight supplemented with metal-halide lamps), air humidity around 50–60 % and...
air CO₂ concentration around 400 µL L⁻¹ with a LI-6200 portable photosynthesis system (LiCor Inc. Lincoln, NE, USA). Midday LWP was measured, with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA), on the leaf for gₛ, A and A measurements.

ABA assay

This was only done in expt I. Pod samples were finely ground under liquid nitrogen, extracted in glass-distilled water using about 1-5 mL per 50 mg f. wt, and extracted on a shaker at 4 °C overnight. The extracts were centrifuged and supernatants assayed for ABA using an enzyme linked immunosorbent assay (ELISA) with monoclonal antibody (AFRC MAC 252) according to Asch et al. (2001). No cross-reaction of the antibody with other compounds in crude extracts was detected when tested according to Quarrie et al. (1988).

Data analysis and statistics

Data were subjected to analysis of variance (ANOVA); appropriate standard errors of the means were calculated. Turkey’s Studentized Range (HSD) Test (P ≤ 0.05) was applied to compare the pod set percentages from different water treatments (SAS Institute, 1988). In expt I, the relationships of pod set to A and to pod [ABA] were evaluated using PROC NLIN of PC SAS (SAS Institute, 1988) with a linear–plateau model, threshold values for A and pod [ABA] at which pod set decreased were obtained (Liu et al., 2004).

RESULTS

LWP

In expt I, at sampling (4 DAA), midday LWP was −0.58 MPa and similar in DS1–DS3 and WW plants. LWP decreased to −1.68 MPa in the most severely droughted (DS6) plants (Fig. 1). In expt II, midday LWP of WW plants was −0.52 MPa, it decreased significantly in DS plants to −1.88 MPa (Fig. 1). ABA application did not affect LWP in WW plants, but significantly increased it to −1.45 MPa in DS plants. BA application had no significant effect on LWP in WW plants, but decreased it to approx. −2.29 MPa in DS plants (Fig. 1).

Pod set

In expt I, pod set of the tagged flowers in WW plants was 66%, similar to DS1–DS3 plants, it decreased in DS4 and was 41% in DS6 plants (Fig. 4). In expt II, pod set of WW plants was 62%, and was decreased significantly to approx. 44% in DS plants (Fig. 4). Exogenous ABA decreased pod set in WW plants to approx. 55%, while it increased it to approx. 49% in DS plants. BA application significantly increased pod set in WW plants to approx. 69% but in DS plants, BA slightly decreased pod set although not significantly (Fig. 4).

Relationships of pod set to A and pod [ABA]

To assess the possible associations between pod set and A and pod endogenous [ABA], regression analyses were performed. In expt I, the relationships of pod set to A and pod [ABA] were well described by linear–plateau functions (Fig. 5). Pod set decreased when A (Fig. 5A) had decreased
by approx. 40% and pod [ABA] (Fig. 5B) had increased 1.5-fold compared with the WW plants; beyond the thresholds, pod set correlated positively with A and negatively with pod [ABA] (Fig. 5). In expt II, a close linear relationship between pod set and A was obtained across the treatments (Fig. 5A).

**DISCUSSION**

**Role of photosynthate supply in regulation of pod set**

Charles-Edwards et al. (1986) suggested that the number of seeds per plant of soybean is linearly correlated with A. This hypothesis was supported by the work of Egli (1988), Egli and Yu (1991) and Jiang and Egli (1993) using source–sink manipulations. However, none of these studies considered the effects of drought stress on photosynthesis and its relationship to pod set of the plants. In the present study, pod set decreased in DS3–DS4 when A had decreased by 40%; beyond this point pod set correlated positively with A (Fig. 5A). This indicates that pod set is insensitive to a decrease in A until a threshold. As in maize
Role of ABA in regulation of pod set

In cereals, several lines of evidence have suggested that drought-induced large concentrations of ABA in the reproductive structures exert a negative effect on fruit/seed set (Ober et al., 1991; Westgate et al., 1996; Setter et al., 2001). In soybean, recent studies showed that [ABA] in flowers and pods was increased by DS, and was associated with a reduction in pod set. Based on these findings, it was speculated that drought-induced an increase in [ABA] causing pod abortion (Liu et al., 2003). However, direct cause and effect could not be shown, and direct evidence to support this hypothesis is still lacking. In the present study, endogenous [ABA] in pods from plants at different stresses have been related to pod set. ABA was applied simultaneously to the plants grown under both WW and DS conditions to test if it could mimic the effect of drought stress on pod set. As expected, pod set correlated negatively with pod [ABA] when the latter was above a threshold value (Fig. 5B); this is in line with early findings in wheat (Westgate et al., 1996). Also, exogenous ABA reduced pod set in WW plants (Fig. 4), in accordance with data for maize (Mambelli and Setter, 1998) and wheat (Wang et al., 2001), suggesting a role of ABA in controlling pod set in soybeans. However, it was also observed that ABA application had a positive effect on pod set in the DS plants. The reason for these contrasting effects of exogenous ABA on pod set in WW vs. DS soybeans is not known. However, it raises the question as to whether ABA affected pod set directly via the processes within the ovary (i.e. cell division) or indirectly via influencing the availability of photosynthate. A similar argument had been raised previously for seed abortion in wheat (Waters et al., 1984). The results of the present study showed clearly that the effect of exogenous ABA on pod set was due partially to its effect on photosynthesis (Fig. 5A). It was clear that exogenous ABA induced closure of stomata in the DS plants at early stages of soil drying, so slowing development of water deficits and resulting in a high LWP, with the result that DS plants could maintain a greater A than those without ABA treatment. Studies have shown that a large LWP is vital for seed set in wheat (Dembinska et al., 1992) and pod set in soybean (Liu et al., 2004).

However, as exogenous ABA decreased pod set in WW plants to an extent greater than expected based on its effect on A as observed in expt I (Fig. 5A), it is suggested that ABA has a direct effect on metabolic processes within the ovary, although the underlying biochemical mechanisms need to be elucidated further.

Role of exogenous BA in regulation of pod set

Under WW conditions, studies in soybean and lupin have shown that the endogenous [CKs] in the pods are positively correlated with the probability of pod set (Emery et al., 2000; Kokubun and Honda, 2000). In the present study, it was observed that exogenous BA increased pod set in WW soybeans, in line with earlier findings (Peterson et al., 1990; Nagel et al., 2001). Based on these, it is suggested that CKs play a role in controlling pod set in soybeans. Studies in soybean (Reese et al., 1995), lupin (Ma et al., 1998), rice (Yang et al., 2002), wheat (Lejeune et al., 1998) and maize (Guo et al., 1995) have indicated that exogenous CKs increase pod/grain set as the hormones increase the availability of current photosynthate and sink strength, and redirect movement of photosynthate into treated tissues. In the present study, it was obvious that exogenous BA increased A in WW plants, which leads to a greater availability of photosynthate for pod growth, and may thereby increase pod set (Fig. 5A).

However, according to the results obtained in expt I, one would expect that increase of photosynthate supply above a critical value would not increase...
pod set. Thus, a direct effect of the hormone on the processes within the ovary (e.g., cell division) may exist.

It was surprising that BA application failed to prevent pod abortion in the DS plants, as expected, by inhibiting the effect of ABA. This was in contrast to our expectation, and earlier findings in DS lupin (Palta and Ludwig, 1997). Radin et al. (1982) demonstrated that application of BA can decrease the sensitivity of stomata to ABA during soil drying. As a consequence of these, exogenous application of BA to the DS plants may lead to a greater loss of water from leaf tissue during mild soil water deficits, resulting in early wilting (Nagel et al., 2001). This may override the positive effect of BA on reproductive development and prevent an increase in pod set under low LWP. Consistent with this, it was observed that exogenous BA decreased LWP and A in DS plants which might have contributed to decreased pod set. However, it was noteworthy that when BA was sprayed on the whole canopy, most of the BA applied was likely to attach to the leaves, and affect the leaf processes, much more than to the pods. This may help to explain why foliar spray of BA did not prevent pod abortion in the DS plants. Therefore, future studies should be based on organ- or tissue-specific application of BA in soybeans to illustrate the underlying mechanisms of CKs in controlling pod set.

It is concluded that drought-induced decrease of current photosynthate supply is significant in inducing pod abortion in soybeans during early reproductive development. ABA and BA affect pod set partially due to their effects on A.

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LITERATURE CITED


