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Effects of Epibrassinolide and Abscisic Acid on Sorghum Plants Growing under Soil Water Deficit

I. Effects on growth and survival*

Hui-lian Xu**, Atsuhiko Shida, Fumio Futatsuya and Atsuhiko Kumura
(*Ageo Laboratory, Nippon-Kayaku Co. Ltd., Saitama 362, Japan*)
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Abstract : Spray treatment with 22R, 23R epibrassinolide (EBR, 0.1 ppm) increased the length and dry weight of both the top and root of sorghum plants under various soil water conditions, although the extent of growth promotion was small. Under well-watered conditions, abscisic acid treatment (ABA, 50 ppm) inhibited top growth but promoted root growth. However, under water-stressed conditions, ABA-treated plants grew at a higher rate than control and EBR-treated plants. The accelerated growth of ABA-treated plants was apparent both in the top and the root. Treatment with 0.1 ppm EBR + 50 ppm ABA inhibited growth even more than ABA treatment under well-watered conditions, but it increased the growth rate more than ABA alone under water stressed conditions. Treatment with EBR + ABA promoted root growth to a larger extent even than ABA treatment. From the above results, it was concluded that EBR increased the effects of ABA both under well-watered and water stressed conditions.

Treatment with EBR and ABA increased the survival ability of plant and leaf under severe drought. This effect of ABA was also enhanced by EBR. Thus, synergistic interaction between ABA and EBR was observed in almost all aspects examined.
Key Words : Abscisic acid, Brassinosteroid, Drought resistance, Epibrassinolide, Growth, Root, Sorghum.

エピブラシノライドおよびアブシジン酸が土壌水分欠乏下に生育するソルガムに及ぼす影響　第1報　生長および生存に及ぼす影響：徐会連・志田篤彦・二谷文夫・玖村敦彦（日本化薬上尾研究所）

要　旨：異なる土壌水分条件下で生育したソルガムの幼植物に，各ポットの4個体を一組として次の薬液を散布した：(1) 22 R, 23 R エピブラシノライド (EBR) 0.1 ppm, (2) アブシジン酸 (ABA) 50 ppm, (3) EBR 0.1 ppm＋ABA 50 ppm. 結果は以下の通りであった.

1) EBR 処理は，すべての水分条件下で乾物生長を促進した．このことから EBR はいろいろな水分条件下で生長促進効果を示すと見られたが，この効果の程度は小さかった．2) ABA 処理は，良好な水分条件下で，地上部乾物重を減少させたが，根の乾物重を増加させた．いっぽう水ストレスがかなり続いた場合，および水ストレスが続いて後，良好な水分条件下に戻された場合には，ABA 処理区の個体乾物重は逆に対照区のそれよりも大きくなった．このことから，ABA は水ストレスによる生長抑制を軽減し，再給水後の生長回復を速めることがわかった．3) 良好な水分条件下で EBR＋ABA 処理は個体乾物重を減少させた．この減少の程度は ABA 単独処理の場合よりもいっそう著しかった．しかし，強度の水ストレス下で，或は強度な水ストレスが長く続いた後，良好な水分条件下に移された場合には，個体乾物重を増加させた．この増加の程度は ABA 単独処理の場合よりも大きかった．4) 以上から EBR と ABA とは顕著な相互効果を示すことがわかった．要するに，ABA はソルガムの生長に対し，水分条件によって正負両様の効果を示し，EBR はこの正負の効果をともに拡大したといえる．5) EBR および ABA による処理がそれぞれ耐乾燥性を高めること，両者の併用によりその効果がいっそう大きくなることは，強度の水ストレス下での葉身の生存率および個体の生存率からも認められた．

キーワード：：アブシジン酸，生長，ソルガム，耐乾燥性，根，ブラシノライド，水ストレス．

As is well known, crop production is seriously affected by various environmental stresses, for example, high and low temperatures, soil water deficit, soil salinity, soil acidity, toxic heavy metals and so on. Of these stresses, drought is the most widespread in the world and affects crop production the most significantly, especially in recent years. Therefore, it becomes most important to increase drought resistance of plants in many and wide areas in the world. One possible way to solve this

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** Present address : Department of Phytology, Horticulture Research Center, Laval University, Quebec, Canada. G1K 7P4.

problem is through appropriate plant growth regulators. Of those so far known, abscisic acid is reported to be effective in increasing drought resistance by both physiological and morphological changes[2,4,5,6,7]. The physiological response of ABA-treated plants to water stress is manifested in diminished stomatal transpiration and strengthened osmotic adjustment[5,6]. The morphological response is apparent in the developed root[8], small-sized cells and xeromorphically-changed structural characteristics of leaf surface[6].

It is reported that brassinosteroids increase resistance or tolerance of plants to low temperature[3], herbicide toxicity[3] and soil salinity[1]. From these facts it is suggested that brassinosteroids can generally increase resistance or tolerance of plants to stresses, though there is little or no evidence for increase in drought resistance[3]. Thus, the authors examined the effects of ABA and 22R, 23R epibrassinolide (EBR), one of the brassinosteroids, on the growth of sorghum plants under various soil water regimes to see whether they could be used as plant regulators in increasing drought resistance in crops.

Materials and Methods

In the present paper the authors focused on the effects of ABA, EBR and their combination on 1) growth rate under various levels of soil water deficit (Exp. I) and 2) survival under very severe soil water deficit conditions (Exp. II).

1. Experiment I

(1) Plant materials

Seeds of grain sorghum (*Sorghum bicolor* (L.) Moench cv. Lucky) were sown in 1/5000 a Wagner pots filled with loamy soil on June 22, 1989. Compound fertilizer ($N : P_2O_5 : K_2O =$ 16 : 14 : 12) was applied at a rate of 2 g per pot. The plants were grown in a natural-light greenhouse, in which air temperature and humidity were not kept constant (maximum temperature, ca 36°C ; minimum relative humidity, 40%). On June 27, 1989, when the seedlings reached the three-leaf stage, they were thinned out with 4 plants of similar size remaining per pot.

(2) Soil water regime

The pots were divided into five groups, i. e., SWR-I to SWR-V. The illustration of the soil water regimes is shown in Fig. 1. The characteristics of the soil water conditions and plant growth were as follows :

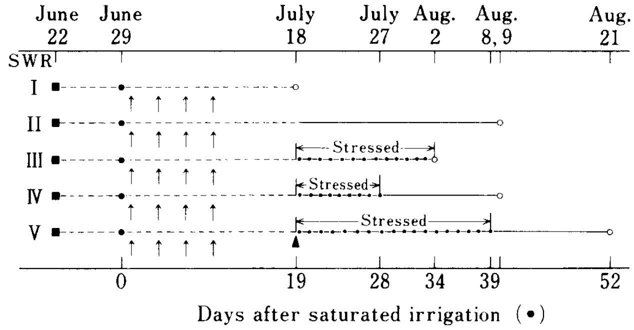

Fig. 1. Experimental conditions in Experiment I.
------ ; No watering but plants experienced no water stress,
—•— ; No watering and plants experienced water stress,
—— ; Watering every two days.
■ ; Sowing, ● ; Saturated irrigation,
○ ; Sampling, ↑ ; Spraying treatment,
▲ ; Beginning of wilting of leaf blades.

SWR-I : After sufficient watering on June 29, plants were not supplied with water until the final sampling on July 18. However, they did not show any sign of wilting during this period, since the seedlings were still small and so water consumption was slight. Thus plants experienced little or no water stress.

SWR-II : Plants were not supplied with water until July 18, but they did not show any sign of wilting during this period. On July 18, when plants showed slight wilting, they were supplied with water and thereafter grew under sufficient water conditions until August 9, when they were sampled. Under this water regime, plants grew practically without water stress throughout the experimental period. Plants in this water regime grew the most vigorously of the five water regimes.

SWR-III : After June 29, the plants were not supplied with any water until the end of the experiment (August 2). Consequently, the plants experienced severe drought in the latter half of the experimental period.

SWR-IV : After the first wilting (July 18), plants were not supplied with water for a period and then they were watered sufficiently until the end of the experiment (August 9).

SWR-V : After the first wilting (July 18), plants were not supplied with water for about three weeks. Consequently, plant growth was strongly depressed. After rewatering on

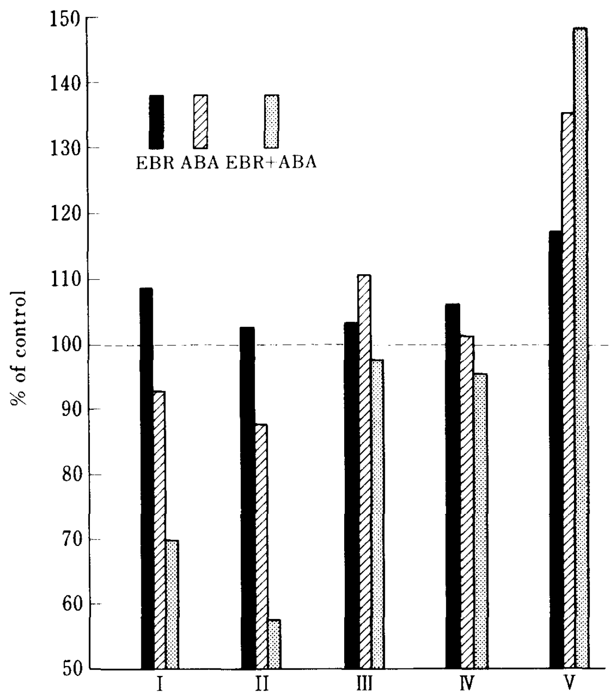

Fig. 2. Effects of EBR, ABA, and EBR+ABA treatments on biomass of sorghum plants under different soil water regimes (Exp. I).
I-V correspond to I-V in Fig. 1.

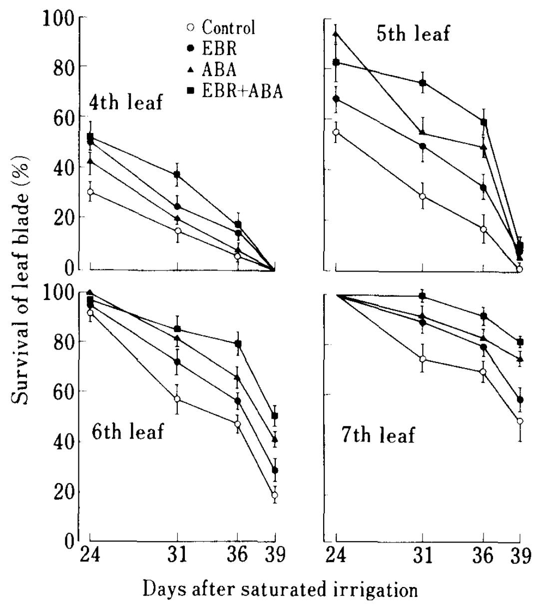

Fig. 3. Survival of leaves of EBR, ABA, and EBR+ABA treated and control plants (Exp. II, Case 1).
Percentage survival of leaf blade was examined in 10 plants of each treatment.

August 8, the plants began to regrow gradually.

(3) **Treatments of ABA and EBR**

The 4 plants in the same pot were treated as one set and sprayed with 1) water (control), 2) EBR (Nippon-Kayaku Co. Ltd.) 0.1 ppm, 3) ABA (Tokyo Kassei Industry Co. Ltd.) 50 ppm, and 4) EBR 0.1 ppm+ABA 50 ppm, respectively. This method of treatment was adopted to compare the effect on plants under exactly the same soil water conditions. "Singlamine" (Sankyo Co. Ltd.) was used as a wetting agent for the treatments, including the water control. Spraying was conducted four times to make sure of the effect, that is, on June 30, July 3, 6, and 9 in all of the soil water regimes. Sufficient solution was spayed onto the plant surface until the leaves were wet completely and the solution ran down along the leaf surface.

2. Experiment II

Experiment II was conducted to examine the effects of ABA and EBR on the survival of sorghum plants under two instances of severe drought conditions.

In case 1, the growing procedure and the soil water regime were the same as those of SWR-V in Exp. I. In case 2, the period of no water supply was prolonged by 5 days. In other aspects, there was no difference from Case 1.

Survival of leaf blade (%) was represented by the proportion of green area to the total estimated on sight. Death or survival of a plant was judged from appearance of the plant after rewatering. When new young leaves began to grow, the plant was regarded as surviving and if they did not, as dead.

Results

1. Experiment I

The results of Exp. I are shown in Table 1 and Fig. 2.

(1) **EBR**

Spray treatment with EBR increased length and dry weight of both top and root in all of the soil regimes. In general, however, the extent of growth promotion was not large, with the largest growth observed in SWR-V.

(2) **ABA**

Under well watered conditions (SWR-I), ABA inhibited growth as expressed in the dry weight of whole plant, but promoted root growth and, as a consequence, increased the root top ratio. In contrast, ABA promoted growth in terms of both length and dry weight

Table 1. Effects of epibrassinolide (EBR) and abscisic acid (ABA) on growth of sorghum plants at vegetative stage under various soil water conditions (Exp. I).

Water condition and sampling	Treatment	Length			Root number*	Dry matter			
		Top (cm)	Root* (cm)	R/T (%)		Top (g)	Root (g)	Total (g)	R/T (%)
I : No stress,	Control	73^{A}	45^{A}	62^{A}	9.0^{A}	0.75^{A}	0.205^{a}	0.96^{A}	28^{A}
sampled at the	EBR	75^{A}	56^{B}	75^{Ba}	9.6^{A}	0.81^{A}	0.236^{b}	1.04^{AB}	30^{A}
end of four	ABA	65^{B}	55^{B}	84^{BCb}	10.1^{B}	0.63^{B}	0.246^{b}	0.89^{B}	39^{B}
time treatments	EBR+ABA	59^{C}	53^{B}	91^{C}	9.2^{A}	0.44^{C}	0.229^{ab}	0.67^{C}	53^{C}
II : Rewatered	Control	130^{Aa}	40^{ab}	30^{ab}	20.3^{ab}	9.16^{ABa}	1.871^{a}	11.03^{ABa}	20^{a}
after treatment	EBR	142^{Ab}	44^{a}	27^{b}	23.3^{a}	9.16^{ABa}	1.871^{a}	11.03^{ABa}	20^{a}
and sampled 21	ABA	131^{A}	43^{ab}	33^{ab}	19.7^{ab}	8.02^{Bb}	1.639^{ab}	9.66^{ab}	22^{a}
days after	EBR+ABA	115^{B}	39^{b}	34^{a}	20.4^{b}	4.92^{C}	1.473^{b}	6.39^{C}	21^{a}
III : Under water	Control	79^{ABa}	55^{A}	68^{a}	11.1^{a}	2.30^{a}	0.555^{A}	2.85^{ac}	25^{a}
stress for 15	EBR	85^{Ab}	61^{B}	72^{ab}	11.8^{a}	2.41^{a}	0.567^{A}	2.97^{a}	24^{a}
days and then	ABA	77^{aBb}	61^{B}	80^{bc}	11.0^{a}	2.40^{a}	0.757^{Ba}	3.16^{b}	32^{b}
sampled	EBR+ABA	75^{B}	55^{A}	78^{c}	11.6^{a}	2.09^{b}	0.690^{Bb}	2.78^{c}	31^{b}
IV : Stressed for	Control	120^{a}	39^{a}	32^{a}	17.5^{Aa}	6.37^{ac}	1.183^{a}	7.55^{a}	19^{ab}
9 days, rewatered	EBR	128^{b}	45^{b}	35^{a}	17.6^{Aa}	6.85^{b}	1.257^{a}	8.11^{b}	18^{a}
then sampled 12	ABA	130^{b}	41^{a}	32^{a}	18.4^{Ab}	6.48^{ab}	1.247^{a}	7.73^{ab}	21^{ab}
days after	EBR+ABA	121^{a}	43^{ab}	36^{a}	19.7^{Bc}	5.98^{c}	1.280^{a}	7.26^{a}	22^{b}
V : Stressed for 21	Control	49^{A}	17^{a}	38^{a}	13.5^{a}	1.84^{Aa}	0.477^{a}	2.32^{Aa}	26^{a}
days, rewatered	EBR	69^{ABa}	26^{ab}	36^{ab}	15.8^{b}	2.11^{A}	0.622^{ab}	2.73^{A}	26^{a}
and sempled 13	ABA	87^{Bb}	29^{b}	34^{bc}	17.8^{c}	2.48^{Ab}	0.683^{b}	3.16^{Ab}	30^{b}
days after	EBR+ABA	97^{Bc}	31^{b}	32^{c}	17.7^{c}	2.64^{B}	0.709^{b}	3.44^{B}	30^{b}

* Root length is the mean of total nodal roots and root number is for total nodal roots. 20 plants of each treatment were examined. The superscript with different small letters means significant difference at $P \leq 0.05$ level and that with different capital letters, at $P \leq 0.01$ level.

I—V here corresponds to I—V in Fig.1. The developmental stages at sampling were: I and III, the 6th leaf stage; II and V, the 10th leaf stage. II, the 8th leaf stage.

in the plants that experienced drought (SWR-III and V). Under SWR-V, where the plants experienced severe drought over a long period, the extent of promotion was especially large.

(3) EBR+ABA

Under well watered conditions (SWR-I and II), treatment with EBR+ABA resulted in a depression in growth which was much larger than that by ABA alone. In plants that experienced moderate (SWR-III) and a short (SWR-IV) drought, the depression was very small. In SWR-V, where the plants experienced a long and severe drought, growth was greatly promoted.

2. Experiment II

(1) Degree of survival of plants

In Case 1 and Case 2, treatment with EBR and ABA seemed to increase the drought resistance of plants. In both cases, the effect was larger in ABA than in EBR, and the largest in ABA+EBR (Table 2).

(2) Survival of leaves

The degree of survival of leaves of plants under a long and severe drought is shown in Fig. 3. From this, it is apparent that treatments with EBR and ABA delayed death of the leaves considerably. The extent of the effect was larger in the ABA-treated plants than in the EBR-treated plants, with the exception of the 4th leaf. The treatment with EBR+ABA showed the largest effect in all of the four leaves.

Discussion

As described in the section of Results, ABA inhibited plant growth in soil regimes where there was no water stress. In contrast, it promoted growth or delayed death of a plant or individual leaves in plants which experienced severe drought. Thus, the effect of the

Table 2. Effects of epibrassinolide (EBR) and abscisic acid (ABA) on survival of sorghum plants under severely water stressed conditions (Exp. II).

Treatment	Survival of plant (Plant/plant)	
	Case 1	Case 2
Control	7/10*	3/5
EBR	9/10	4/5
ABA	10/10	5/5
EBR+ABA	10/10	5/5

* This means seven of ten plants still alive when they were rewatered after the drought period.

ABA treatment varied essentially depending on soil water regimes.

EBR applied alone delayed death of plant or individual leaves under severe drought to an extent smaller than that of ABA (Table 2 and Fig. 3). As for the effect on plant growth, EBR singly applied promoted growth to a small extent in all the soil regimes, including well watered and severe drought conditions. However, when EBR was combined with ABA, the effect was different from that of EBR singly applied. That is, EBR greatly accelerated the inhibitive effects of ABA under well watered conditions. On the other hand, EBR accelerated promotive effects of ABA under severe drought conditions.

Thus it seems that EBR strengthened both the inhibitive and promotive effects of ABA, which were observed under well-watered and drought conditions respectively, though EBR itself had a weak promotive effect always. From this it can be said that the combined effect of ABA and EBR is quite synergistic.

It has been reported that concentration of endogenous ABA increases under drought conditions and exogenous ABA gives plants xeromorphic characteristics[6]. The present paper showed that ABA increased drought resistance in terms of both growth rate and survival ability under severe drought conditions. EBR strengthened this effect of ABA.

The mechanism for the increase in drought resistance by ABA and ABA combined with EBR will be examined in the next paper. What can be concluded from these results is the promotion of root growth. Just after four spray treatments by ABA, growth of root relative to that of top was greatly increased by the treatment as shown in R/T ratio, both in length and dry matter (Table 1, SWR-I). ABA combined with EBR showed the same effect to a further degree. The promotion of root growth as above might, at least in part, account for the drought resistance of plants under drought conditions in the later period of growth.

The spraying of ABA combined with EBR was effective in increasing drought resistance. However, the same treatment showed a large inhibitive effect under well-watered conditions. This creates a difficult problem in applying these plant regulators in areas where reliable prediction of water conditions in crop-growing season can not be expected.

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