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FLOWERING AND POD FORMATION RESPONSE TO METRIBUZIN HERBICIDE AND NACI STRESS AND WITH CORRELATION TO CHLOROGENIC ACID APPLICATION IN *Phaseolus vulgaris*

Hasaneen, M. N. A.
Dept. of Botany, Faculty of Science, Mansoura Univ., Mansoura, Egypt.
E-mail: Mohammednagib@mans.edu.eg

ABSTRACT

Reproduction parameters (number of Flowers, number of pods, pod fresh and dry weight, content of Flower-induction (Phenylpropanoid) chlorogenic acid (CGA) of french bean plants supplemented with 0.5 ug.g^{-1} and 2.5 ug.g^{-1} of metribuzin either alone or incombination with 50 ug.g^{-1} NaCl, were increased significantly, whereas 5 ug. g^{-1} and 10 ug.g^{-1} of metribuzin either alone or in combination with 50 ug. g^{-1} NaCI, gave rise to significant decrease in all reproduction parameters. CGA administration to such media induced significant increases in all reproductive parameters determined. On the other hand, CGA content of french bean plants treated with 5 ug. g^{-1} CGA showed significant increase at 0.5 and 2.5 ug. g^{-1} metribuzin either alone or in combination with NaCI whereas 5 ug.g^{-1}CGA administered to 5 and 10 ug. g^{-1} metribuzin alone or incombination with NaCl a significant decrease in CGA content was apparent at either flowering or fruiting stages.

Keywords: Chlorogenic acid (CGA), metribuzin, NaCI stress, solution culture, *Phaseolus vulgaris*.

INTRODUCTION

Floral induction under environmental stresses (poor nutritional conditions, water stress, herbicide stress, temperature stress), is closely correlated with the accumulation of phenylpropanoids such as chlorogenic acid (CGA), pinoresinol-B-D-glucoside (PRG) and p-coumaroylquinic acid (COQ) (HIRAL el al., 1993). Seedlings of *Pharbitis nil* strains violet, a short-day plant, are induced to flower even in continuing light by culture under conditions of poor nutrition, high intensity light and low temperature (SHINOZAKI AND TAKIMOTO, 1982). HIRAL *el al*. (1993) reported that the content of CGA was increased in the cotyledons of *Pharbitis nil* seedling grown under stress conditions.

In the present study, levels of CGA in french bean plants were examined in relation to flowering and podding under berbicide and/or salt stress conditions. The effects of CGA treatment on flower induction of French bean plants under such stress conditions were also examined to confirm the correlation.

MATERIALS AND METHODS

Homogenous seeds of french bean (*Phaseolus vulgaris* var. contender) were sterilized and germinated on Whatman No. I filter paper watered with 20 cm^3 of Hoagland nutrient solution (1/4 -strength) in plastic dishes. The germinating dishes of french bean were incubated in the dark at

25 'c for 4 days. Then 5 uniform germinating seeds were placed in a perspex plate suspended over a black-painted glass cylinder (1000 cm^3) containing either Hoagland nutrient or Hoagland nutrient solution supplemented with herbicide and/or NaCl (Hasaneen *et al.,* 1994). The cylinders were placed in a growth chamber adjusted at optimum growth conditions (temperature 28 °C ± 2 °C, light intensity 3000-3500 lux, relative humidity 60-70% and continuous aeration from air pump at a rate 2 L/h/cylinder; STEINGROVER, 1983). The culture solution of each respective treatment was replaced twice a week (STEINGROVER, 1983). After 30 days from planting, all samples were treated with chlorogenic acid (5 $ug.g^{-1}$). Sampling of flowers and fruits were performed after 40 and 60 days respectively.

Determination of chlorogenic acid

According to HIRAI *et al.* (1993), a known fresh weight of french bean plants at flowering and fruiting stages, were boiled in 5 cm^3 of 2% acetic acid for 10 minutes, a 20 ul portion of the extract was analyzed by HPLC with MS pack CI8 (4.6 mm i.d.x 150 mm, M and S Instruments trading inc.). Solvent A (92% water, 5% methanol, 1% n-butanol and 2% acetic acid), solvent B (97% methanol, 1% n-butanol and 2% acetic acid) were used for elution as follows: 0-10 min. with 0% B, 10-20 min. with 10% B, 20-30 min. with 20% B, 30-40 min. with 30% B and 40-50 min. with 40% B. The flow rate was 1.0 $ml.min^{-1}$ and absorbance at 280 nm was measured.

RESULTS AND DISCUSSION

Changes in reproductive parameters

Treatment of french bean plants with 0.5 $ug.-g^{-1}$ and 2.5 $ug.g^{-1}$ of metribuzin either alone or in combination with .50 $ug.g^{-1}$ NaCI induced significant increase in reproductive parameters, i.e. number of flowers, number of fruits, fresh and dry weight of pods (Table 1). On the other hand, 5 $ug.g^{-1}$ and 10 $ug.g^{-1}$ of metribuzin +50 $ug.g^{-1}$ NaCl led to significant decreases in the number of flowers, and fruits. The changes were more pronounced in samples treated with herbicide alone than in those supplemented with herbicide in combination with NaCI.

Inclusion of CGA into culture media containing 0.5 and 2.5 $ug.g^{-1}$ herbicide either alone or in combination with NaCI induced further increase in reproductive organs (number of flowers, and fruits). On the other hand, CGA administration in 5 and 10 $ug.g^{-1}$ metribuzin alone or +50 $ug.g^{-1}$ NaCI induced significant increase in the number of flowers, and fruits (Table 1).

El-Maghraby (1997) observed significant decrease in the various growth parameters in addition to the number of flowers, number of pods and their water content, at flowering and fruiting stages of french bean plants treated with different concentrations of stomp herbicide. Gross morphological effects of metribuzin either alone or in combination with 50 $ug.g^{-1}$ NaCI on reproductive parameters (Table 1) may suggest abnormal cytological behavior. The effects of the herbicide were reported as preventative cell wall formation, elongation of cells and extension replication of nuclei (Nemat-Allah, 1991; Hasaneen *et al.*, 1994; El-maghraby, 1997). They hypothesized that a primary effect of different herbicidal treatments in different plants are on cell division and that this may be the mechanism of herbicidal action.

Table 1. Effect of CGA on flowering and fruiting responses in french bean plants stressed with metribuzin and/or NaCl. Values with *and **are significantly different at p-levels 0.05 and 0.01 respectively (LSD test)

	Concentration		Flowering		Fruiting	
Metribuzin ($ug.g^{-1}$)	NaCl ($ug.g^{-1}$)	CGA ($ug.g^{-1}$)	Mean flower No. / plant	Mean pod No./plant	Mean pod fresh weight (g)	Mean pod dry weight (g)
0.0	0	0	4.1	2.1	1.42	0.121
0.5	0	0	4.3	2.6**	1.54**	0.151**
2.5	0	0	4.4*	2.8	1.61**	0.169**
5.0	0	0	3.5**	1.8**	1.32	0.109**
10.0	0	0	2.2**	1.4**	1.01**	0.092**
L.S.D at 5% level	-	-	0.20	0.10	0.07	0.006
L.S.D. at 1% level	-	-	0.31	0.15	0.10	0.009
0.0	50	0	3.8	2.0	1.41	0.120
0.5	50	0	4.0	2.2**	1.43	0.123
2.5	50	0	4.2**	2.3**	1.45	0.124
5.0	50	0	3.5**	1.9*	1.41	0.121
10.0	50	0	3.3**	1.8*	1.31	0.109**
L.S.D at 5% level	-	-	0.19	0.10	0.07	0.006
L.S.D. at 1% level	-	-	0.28	0.15	0.10	0.009
0.0	0	5	4.8	2.6	1.55	0.152
0.5	0	5	5.2**	2.9**	1.65	0.171**
2.5	0	5	5.6**	3.0**	1.71**	0.180**
5.0	0	5	4.8	2.55*	1.52	0.151
10.0	0	5	4.8	2.5	1.51	0.150
L.S.D at 5% level	-	-	0.24	0.13	0.07	0.07
L.S.D. at 1% level	-	-	0.36	0.19	0.11	0.11
0.0	50	5	4.0	2.6	1.56	0.153
0.5	50	5	4.4**	2.8**	1.62	0.169
2.5	50	5	4.8**	3.0**	1.72**	0.180
5.0	50	5	4.0	2.6	1.50	0.150
10.0	50	5	3.9	2.5	1.48	0.147
L.S.D at 5% level	-	-	0.2	0.13	0.07	0.07
L.S.D. at 1% level	-	-	0.3	0.14	0.11	0.11

As consequence of herbicide and NaCI treatment, in the literature, inhibition of protein synthesis (DUKE *et al.,* 1971), hormone regulation (Fedtke, 1982) and pigmentation (ENSMINGER and HESS, 1985) have often been observed. These responses are presumably due to a direct or indirect effect of herbicide and/or salinity on the activity of some enzyme systems.

Treatment of french bean plants with CGA prior to flowering stage induced significant increase in number of flowers and fruits especially in culture media that cause inhibition in flowering and fruiting (5 $ug.g^{-1}$ and 10 $ug.g^{-1}$ metribuzin + 50 $ug.g^{-1}$ NaCI). Floral induction under stress conditions is closely correlated with the accumulation of phenyl propanoids, such as CGA (Shinozaki *et al.,* 1988 a, b & c and Hirai *et al.,* 1993). Therefore, the mechanism of action of CGA action on flowering seems to differ under different conditions.

Correlation between levels of CGA and the flowering response under stress conditions

Table 2 shows the changes in the levels of CGA content in French plants and the flowering response under herbicide and/or NaCI stress. CGA content of french bean plants at flowering and fruiting stages was increased significantly under the influence of herbicide and/or NaCI (0.5 and 2.5 ug.g^{-1}). On the other hand, a significant decreases in CGA content of french bean plants treated with 5 and 10 Ug.g^{-1} metribuzin alone or in combination with 50 Ug.g^{-1} NaCI (Table 2).

The results shown in tables I and 2 reported that metribuzin (5 and 10 ug.g^{-1}) and/or NaCI inhibited flowering and fruiting of french bean plants at the same time as it suppressed the accumulation of CGA. 1n a previous study under poor nutritional conditions (SHINOZAKI *et aL,* 1988 a, b) 100 um aminooxyacetic acid completely inhibited flowering. 'These results may be due to differences in absorption and accumulation of aminooxyacetic acid under stress conditions.

Table 2. CGA content of controls as well as differently treated French bean plants at flowering and fruiting stages. Values with * and **are significantly different at p-levels 0.05 and 0.01 respectively (LSD test)

Concentration			CGA (mg/g fresh weight)	
Metribuzin (ug.g^{-1})	**NaCl (ug.g^{-1})**	**CGA (ug.g^{-1})**	**Flowering**	**Fruiting**
0.0	0	0	10.2	4.6
0.5	0	0	12.3**	5.4**
2.5	0	0	13.0**	6.0**
5.0	0	0	7.6**	3.8**
10.0	0	0	5.3**	2.5**
L.S.D at 5% level	-	-	0.51	0.23
L.S.D. at 1% level	-	-	0.76	0.34
0.0	50	0	7.9	2.8
0.5	50	0	8.4**	3.1**
2.5	50	0	9.2**	3.9**
5.0	50	0	5.3**	2.1**
10.0	50	0	4.5**	1.8**
L.S.D at 5% level	-	-	0.39	0.14
L.S.D. at 1% level	-	-	0.59	0.21
0.0	0	5	16.4	4.8
0.5	0	5	17.2	5.6**
2.5	0	5	18.3**	6.2**
5.0	0	5	10.1**	3.9**
10.0	0	5	8.6**	2.7**
L.S.D at 5% level	-	-	0.82	0.24
L.S.D. at 1% level	-	-	1.23	0.36
0.0	50	5	13.4	2.9
0.5	50	5	14.9**	3.3**
2.5	50	5	15.2**	4.2**
5.0	50	5	8.4**	2.4**
10.0	50	5	7.1**	1.9**
L.S.D at 5% level	-	-	0.67	0.14
L.S.D. at 1% level	-	-	1.00	0.21

The present results suggest that floral initiation under stress conditions (herbicide and/or NaCl) is induced *via* the mechanism of action CGA on flowering which seems to differ under different concentrations. Application of CGA or pinoresinol-B-glucoside (PRG) to the roots of *Pharbitis nil* under stress conditions did not increase the levels of these compounds in the plants (Shinozaki *et al.,* 1988 c). An analysis of the role of these phenylpropanoids in the induction of flowering under stress conditions is now in progress and needs further studies.

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إستجابة التزهير وتكوين القرون في نبات الفاصوليا للمبيد العشبي متربيوزن وملح كلوريد الصوديوم و ارتباطه بإضافة حمض الكلوروجينيك

محمد نجيب عبد الغني حسنين
قسم النبات – كلية علوم المنصورة – جامعة المنصورة – مصر.

تم في هذا البحث كاستكمال لبحوث سابقة – دراسة التزهير وتكوين القرون في نبات الفاصوليا الحمراء نتيجة المعاملة بالمبيد العشبي متربيوزن تحت ظروف الإجهاد الملحي و ارتباط ذلك بإضافة حمض الكلوروجينك أظهرت نتائج البحث أن معدلات الأزهار و الأثمار في نباتات الفاصوليا تزداد بإضافة حمض الكلوروجينك إلى الأوساط الغذائية المستعملة علي محلول هوجولاند مضافا إليه المبيد العشبي متربيوزن بتركيزاته المنخفضة والعالية مضافا إليه ملح الطعام بتركيز 50 ميكروجرام لكل جرام علي الرغم من أن التركيزات المنخفضة من المبيد العشبي تؤدي إلى زيادة في مستويات الأزهار و الأثمار فيما التركيزات العالية من المبيد العشبي منفردا تؤدي إلى نقص معنوي في مستويات الأزهار و الأثمار ومن ناحية أخري لوحظ زيادة محتوي حمض الكلوروجينيك في النباتات المعاملة بالتركيزات المنخفضة من المبيد العشبي متربيوزن (5 ، 2.5 ميكروجرام لكل جرام) فيما في التركيزات العالية من نفس المبيد العشبي (10،5 ميكروجرام لكل جرام) أدت إلى نقص معنوي في محتوي حمض الكلوروجينيك. ولقد تم مناقشة النتائج في ضوء الميكانيكيات المختلفة لتأثير حمض الكلورجينيك علي أزهار و أثمار النباتات وخاصة نبات الفاصوليا النامية تحت ظروف الإجهاد المختلفة.