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Full Length Research Paper

Effect of seed soaking treatment with growth regulators on phytohormone level and sex modification in cucumber (*CUCUMIS SATIVUS* L.)

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Field experiments were undertaken at national agricultural research centre Islamabad, Pakistan during 2004 and 2005 to study the seed soaking effects of 2-chloroethylphosphonic acid (ethrel), gibberellic acid and maleic hydrazide (MH) on sex modification in cucumber and to find out the critical phytohormones level of endogenous hormones for lowest sex ratio. Seeds were soaked in different concentrations of growth regulators for 20 h prior to sowing. Seed soaking with MH at 450 ∝mol/L highly increased the number of pistillate flowers and fruit yield per plant and decreased the sex ratio compared to that of untreated control. The phytohormone levels of indole acetic acid (IAA) at blooming and fruiting stages were significantly higher with MH at 450 µmol/L and had a positive significant correlation with the number of pistillate flower/plant and negative correlation with sex ratio. Fruit yield had also a positive significant correlation with endogenous IAA. Ethrel also significantly lowered the male to female sex ratio in cucumber.

Key words: *Cucumis sativus*, sex expression, ethrel, gibberellic acid and maleic hydrazide.

INTRODUCTION

Sex expression in cucumber (*Cucumis sativus* L.) can be modified effectively with plant growth regulators. The ethrel and maleic hydrazide are remarkably effective in increasing femaleness in cucumber (Kshirsagar et al., 1995; Vadigeri, 2001; Rafeekher et al., 2002) while gibberellins promote formation of staminate flowers (Choudhury and Patil, 1962; Pike and Peterson, 1969). Plant growth regulators have been mostly foliarly applied for the modification of sex expression of flowers in cucumber and very little work has been reported via seed soaking in vegetable crops. Furthermore, the work with growth regulators has mainly examined sex modification in cucumber and to a lesser extent the internal phytohormone level which brings about this change. Pandey et al. (1978) reported that seed treatment with MH at 200 ppm increased the pistillate flowers of *Luffa aegyptiaca* Mill. The cultivar Sialkot Selection of cucumber (*C. sativus* L.*)* which is monoecious has a strong tendency towards maleness. One of the factors limiting yield of this cultivar is the production of relatively low pistillate flowers which results in a high ♂ to ♀ sex ratio. The present work was undertaken to examine the effect of seed soaking with ethrel, gibberellic acid and maleic hydrazide (MH) in regulation of sex expression in a monoecious cultivar of cucumber with emphasis on estimating the level of phytohormones, indole acetic acid (IAA) and gibberellic acid (GA_3) as one of the mechanism controlling sex expression in plants.

MATERIALS AND METHODS

Seeds of cucumber cultivar 'Sialkot Selection' were soaked for 20 hours in the aqueous solution of ethrel at 700, 1750, 2750 and 3800 µmol/L, GA_3 at 15, 30, 45, 60 and 75 µmol/L and MH at 225, 450, 675 and 900 µmol/L before sowing in march 2004 and 2005.

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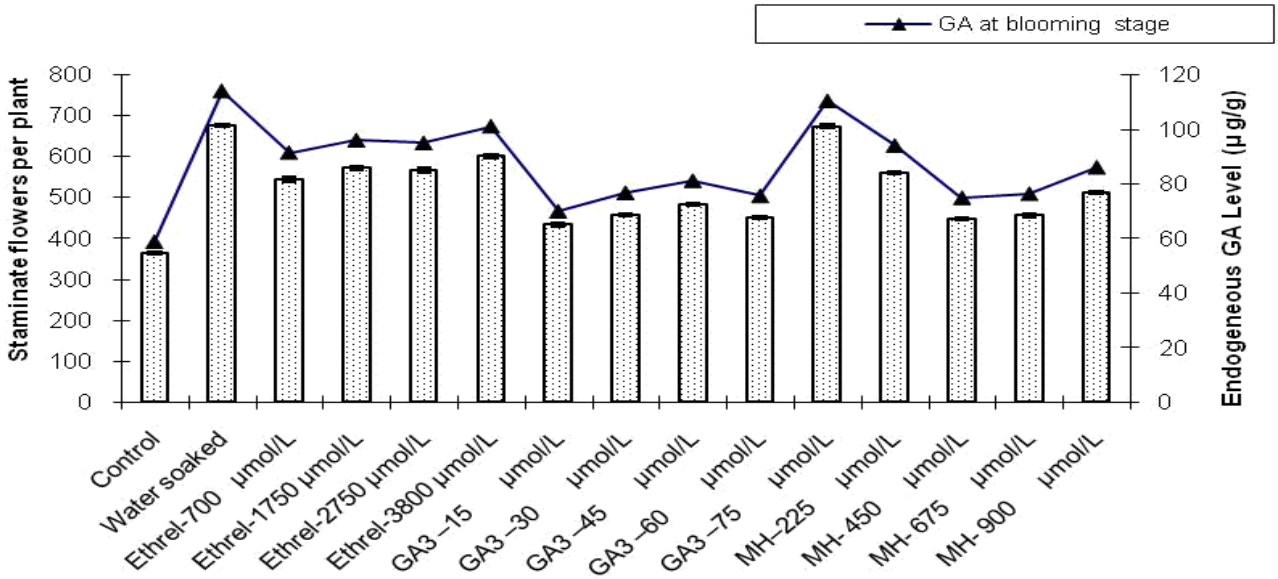

Figure 1. Effect of plant growth regulators on staminate flowers per plant (means of two years with 3 replications per year) and its interaction with endogenous GA_3 level at blooming stage. Vertical bars show the standard error means.

For control, seeds were soaked in distilled water. Beside this there was a field control that is, without soaking. When seedlings attained 1 to 2 true leaf stages, healthy seedlings were transferred to a well prepared open field, laid out as randomized complete block design (RCBD). Plot size was 2.25 × 0.6 m with three replications. Standard production practices recommended for open field crops were followed. The studies were conducted at vegetable crops research programme, national agricultural research centre Islamabad, Pakistan. Seedlings were protected from the attack of red pumpkin beetle by dusting with carboxyl and ash in the ratio of (1:10) at 2 to 4 leaf stage of plant growth. Total number of staminate and pistillate flowers/plant were recorded from the five selected plants/replication/treatment. Sex ratio was calculated by dividing number of staminate flowers with number of pistillate flowers. Total number of fruits/plant were recorded during entire growth period. Single fruit weight (g) was recorded by average of 10 randomly selected fruits/replication. Total soluble solids (TSS) was determined by average of 10 randomly selected fruits/replication with Abby's Hand Refractrometer (ATAGO Co. Ltd. Japan). Chlorophyll content was measured with chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan) at blooming stage (65 DAS) (Arenas et al., 2002). Leaf area index (LAI) was determined using the Leaf canopy analyzer 2000, LI-COR, Inc., 4421 (Welles and Norman, 1991).Yield was recorded as weight (kg) of fruits/plant.

For endogenous phytohormones level the leaves of cucumber plant were collected at four growth stages that is, two leaf (20 DAS), flower initiation (30 DAS), blooming (65 DAS) and fruiting (80 DAS) for the extraction of endogenous GA_3 and IAA. The extraction and purification was made following the method of Kettner and Droffling, (1995). The plant leaves (1 g) were ground in methanol (80%) at 4°C with an antioxidant, butylated hydroxyl toluene (BHT). The leaves were extracted at 4°C in dark for 72 h with subsequent changes of solvent. The extracted sample was centrifuged and the supernatant was reduced to aqueous phase using rotary thin film evaporator (RFE). The pH of the aqueous phase was adjusted between 2.5 to 3.0 and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate was dried down completely using rotary thin film evaporator (RFE). The dried sample was re-dissolved in 1.5 ml of methanol (100%) and was analyzed on HPLC (Agilent 1100) using UV detector and C-18 column (39 × 300 mm). For identification of hormones, 10 µl sample filtered through 0.45 millipore filter was injected into column. Pure IAA and GA (Sigma, USA) were used as standard for identification and quantification of plant hormones. These growth hormones were identified on the basis of retention time and peak area of standards. Methanol, acetic acid and water (30:1:70) were used as mobile phase. The flow rate was adjusted at 0.5 ml/min with an average time of 20 min/sample. The wave length used for the detection of IAA was 280 nm (Sarwar et al., 1992) whereas for GA analysis it was set at 254 nm (Li et al., 1994). The data from each experiment were analyzed using the ANOVA procedures for a randomized complete block design with the treatments randomly assigned in three replications. The graphs were plotted using Microsoft Excel.

RESULTS AND DISCUSSION

Plant growth regulators are involved in sex determination of plants and have effects on modulating the ratio of pistillate and staminate flowers. Galun et al. (1963) concluded that genetic, environmental and chemical factors are involved in the control of stamens and ovary differentiation in cucumber floral bud. Harrison (1957) also reported that sex expression of monoecious cucumber could be modified by application of growth substances.

Number of staminate flowers per plant

All hormonal treatments significantly increased the total number of staminate flowers per plant as compared ($p \leq 0.05$) with the control (Figure 1). This is in contrast to the

Table 1. Effect of seed soaking with plant growth regulators on changes in endogenous phytohormones levels in the leaves of cucumber cv. 'Sialkot Selection'. Seeds were soaked for 20 h prior to sowing in distilled water or different concentrations of growth regulators. Measurements for endogenous hormones were made at different physiological stages.

Treatments (µmol/L)	Endogenous GA_3 level (µg/g)				Endogenous IAA level (µg/g)			
	Two leaf stage	Flower initiation stage	Blooming stage	Fruiting stage	Two leaf stage	Flower initiation stage	Blooming stage	Fruiting stage
Control	30.5^{n}	35.5^{n}	59.0^{o}	41.3^{m}	10.8^{k}	11.2^{l}	11.4^{o}	10.8^{n}
Water soaked	44.9^{e}	51.9^{j}	113.9^{a}	48.1^{b}	11.0^{l}	11.0^{k}	32.9^{n}	27.9^{m}
Ethrel-700	30.8^{m}	32.9^{o}	91.5^{g}	40.2^{n}	35.0^{b}	45.0^{b}	45.7^{j}	40.9^{l}
Ethrel-1750	38.2^{l}	45.3^{l}	96.0^{d}	41.9^{l}	26.3^{d}	36.3^{e}	56.3^{g}	39.3^{j}
Ethrel-2750	39.1^{k}	48.0^{k}	95.0^{e}	44.0^{l}	10.9^{j}	10.9^{l}	38.2^{l}	34.1^{l}
Ethrel-3800	44.9^{e}	44.1^{m}	101.1^{c}	46.4^{d}	10.8^{k}	10.9^{l}	36.3^{m}	35.8^{k}
GA_3 –15	44.4^{f}	68.0^{c}	70.1^{n}	39.3^{o}	17.8^{f}	33.9^{g}	50.5^{n}	50.5^{g}
GA_3 –30	42.7^{n}	63.5^{e}	76.8^{j}	42.3^{k}	16.9^{g}	32.3^{n}	73.5^{e}	71.2^{e}
GA_3 –45	39.8^{j}	53.1^{l}	81.2^{l}	43.1^{j}	11.0^{l}	11.1^{j}	90.5^{c}	78.0^{c}
GA_3–60	40.0^{l}	53.2^{n}	75.7^{l}	45.2^{f}	23.5^{e}	43.0^{d}	100.0^{b}	89.1^{b}
GA_3 –75	42.8^{g}	55.1^{g}	110.3^{b}	44.1^{n}	10.9^{j}	11.0^{k}	47.7^{l}	45.1^{n}
MH–225	55.5^{a}	89.7^{a}	94.0^{f}	47.3^{c}	47.3^{a}	80.2^{a}	83.1^{d}	77.9^{d}
MH- 450	51.1^{b}	73.3^{b}	75.0^{m}	45.9^{e}	26.9^{c}	44.8^{c}	103.4^{a}	91.5^{a}
MH- 675	47.9^{d}	66.2^{a}	76.5^{k}	48.3^{a}	14.2^{n}	35.1^{f}	62.1^{f}	62.0^{f}
MH- 900	48.3^{c}	58.9^{f}	86.1^{h}	44.5^{g}	11.0^{l}	11.2^{l}	41.5^{k}	40.9^{l}
LSD value	0.06335	0.07315	0.2164	0.06335	0.07315	0.06335	0.08959	0.06335

Means followed by the same letter within same columns do not differ significantly at 5 % level.

All growth regulator treatments significantly

findings of Ratnapala and Silva (1989) and Surendranath and Srirama (1981) who reported that ethrel decreased the number of staminate flowers in cucumber. However, maximum number of staminate flowers was observed with water soaked control and GA_3 at 75 µmol/L. Possibly free GA is released from conjugated form which in turn affect the staminate flower production similar to that of GA treatment. Our results also revealed that number of staminate flowers had an interaction with level of endogenous GA_3 at blooming stage (Table 1). Other workers have reported that application of GA_3 increased the number of male flowers in monoecious cucumber (Jutamanee et al., 1994). The effect of year was significant. More number of staminate flowers was produced during 2004 as compared to 2005. This difference might be attributed to the changes in the level of hormones at relatively lower temperature in 2005 as compared to that of 2004 (Table 3). Nitsch et al. (1952) observed development of male flowers only in cucumber under high temperature (30°C).

Number of pistillate flowers per plant

increased total number of pistillate flowers as compared to both water soaked and untreated control (Figure 2) which may be due to high level of endogenous IAA at blooming stage (Table 1). Kobayashi et al. (1989) suggested that IAA may play a role in regulating the reproductive growth of rice. It has been reported that ethrel increased the pistillate flowers in cucumber (Ratnapala and Silva, 1989; Jutamanee et al., 1994; Kshirsagar et al., 1995; Vadigeri et al., 2001). The MH at 450 µmol/l produced the highest number of pistillate flowers/plant (36.3) and had the highest level of endogenous IAA at blooming stage. In addition, as revealed by a significant positive correlation

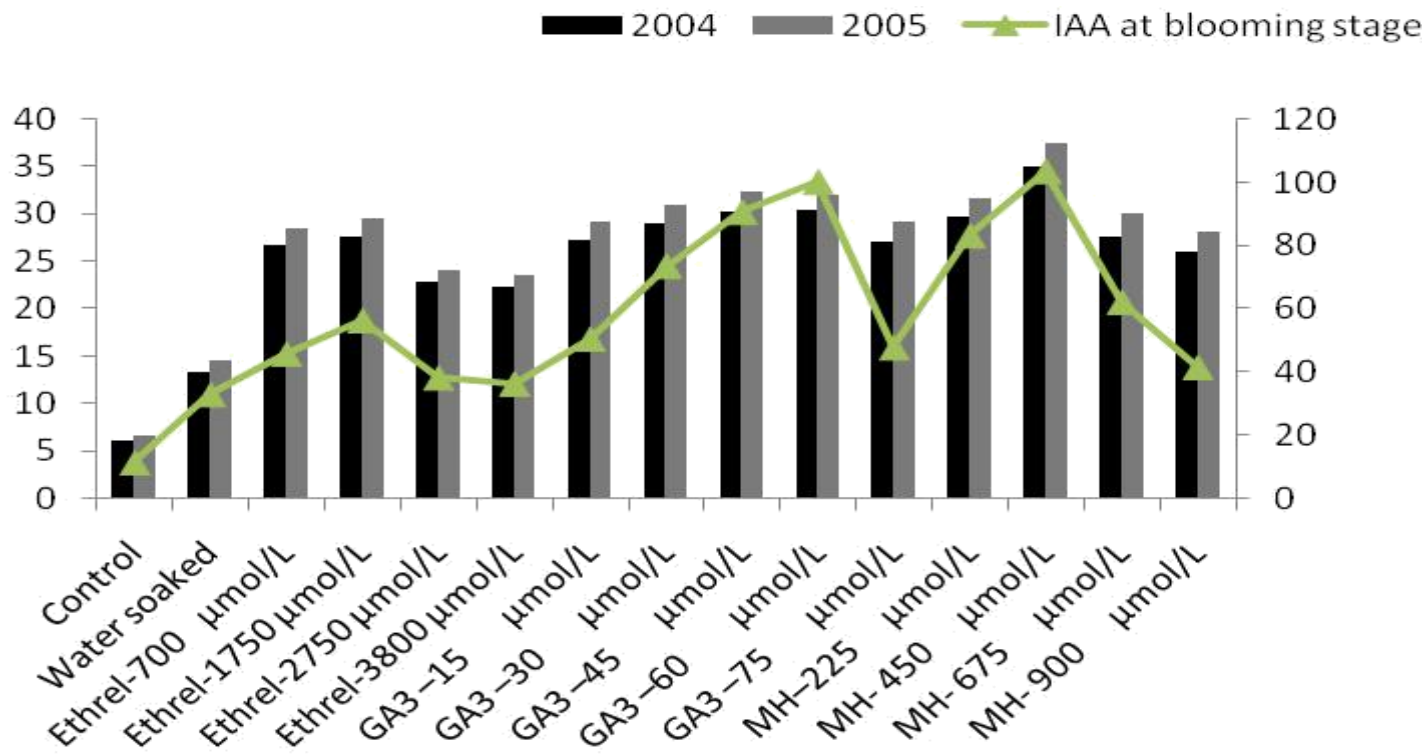

Figure 2. Effect of plant growth regulators on pistillate flowers per plant (means of two years with 3 replications per year) and its interaction with endogenous IAA level at blooming stage. Vertical bars show the standard error means.

(r=0.795) was found between number of pistillate flowers and IAA concentration showing that IAA has a role stimulating flower female differentiation (Table 2). These findings are in agreement with those of Vadigeri et al. (2001) and Kshirsagar (1995) who reported that MH and ethrel enhanced pistillate flowers. Seed soaking in water prior to sowing also resulted in significant increase in the number of pistillate flowers/plant as compared ($p \leq 0.05$) to untreated control. Possibly different hydrolytic enzymes were activated as a result of seed soaking resulting in the release of growth promoting hormones in free form from the stored conjugated form. Pistillate flowers produced were more in number in 2005, this difference might be attributed to modulation in hormone level at relatively lower temperature in 2005 as compared to 2004 (Table 3). Stimulation of pistillate flowers in several varieties of cucumber under low temperature and short day has also been reported (Fukushima et al., 1968).

Sex ratio

Seed soaking with growth regulators significantly lowered sex ratio in cucumber compared with untreated control (Figure 3). Growth regulator treatments increased the number of pistillate flowers which had a significant negative correlation (r= -0.949) with sex ratio (Table 2). Previous reports confirm the change in male to female flower ratio in cucumber by reducing the number of male flowers and increasing the female flowers (Hussain et al., 1990; Kshirsagar et al., 1995; Das et al., 2001; Rafeekher et al., 2002). The lowest male to female flower ratio (12.4) was recorded with treatment of MH at 450 µmol/l followed by GA_3 at 60 µmol/L. IAA level at blooming stage had a negative significant correlation (r= -0.719) with sex ratio (Table 2). Possibly the increased endogenous IAA level might affect sex ratio and ultimately fruit yield by increasing number of pistillate flowers/plant. Water soaking of seeds also lowered sex ratio as compared ($p \leq 0.05$) with untreated control.

TSS

All concentrations of ethrel (except 700 and 1750 µmol/L),gibberellic acid and maleic hydrazide significantly increased the TSS of fruit as compared to water soaked control ($p \leq 0.05$). Maximum increase (3.23 and 3.19 Brix %) was observed with MH at 450 µmol/L and GA_3 at 60 µmol/l followed by GA_3 at 30 and 45 µmol/L (Figure 4). This might be due to higher level of endogenous IAA (Table 1) at fruiting stage. Louis et al. (1952) reported that fruit of Valencia orange trees sprayed in late winter

Table 2. Correlation coefficient for the 'effect of seed soaking with plant growth regulators on sex expression and yield in cucumber.

Correlation coefficient	Sex ratio	Chlorophyll contents	Leaf area index	Total soluble solids	Number of fruits per plant	IAA level at blooming stage	IAA level at fruiting stage	Fruit yield per plant
Number of pistillate flower per plant	-0.949**	-0.315**	0.756**	0.707**	0.710**	0.795**	0.799**	0.689**
Sex ratio		0.294**	-0.804**	-0.658**	-0.698**	-0.719**	-0.740**	-0.658**
Chlorophyll contents			0.210*	-0.217*	-0.218*	-0.245*	-0.286**	-0.279**
Leaf area index				0.523**	0.566**	0.605**	0.597**	0.505**
Total soluble solids					0.596**	0.855**	0.890**	0.710**
Number of fruits per plant						0.634**	0.666**	0.884**
IAA level at blooming stage								0.736**
IAA level at fruiting stage								0.757**

**, * p < 0.01, 0.05, respectively.

Table 3. Meteorological data during the study period .

Period of study	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)
March 2004	29.1	10.4	61.7	0.0
April 2004	31.8	15.6	52.0	93.3
May, 2004	36.3	17.9	39.3	6.6
June, 2004	35.3	21.6	54.1	131.7
August, 2004	32.7	22.0	75.4	258.5
March, 2005	22.9	10.2	80.0	75.4
April, 2005	29.4	12.2	55.2	13.9
May, 2005	33.4	16.8	42.8	20.3
June, 2005	39.3	20.4	45.2	72.6

Source: Metrological section, water resources Research institute (WRRI), NARC, Islamabad.

showed a correlation between the concentration of MH and the total soluble solids. Robbertse and Stassen (2004) reported that growth retardant paclobutrazole increased the fruit TSS in Mango. The minimum TSS (2.62 Brix %) was observed in untreated control.

Single fruit weight

GA_3 at 60 µmol/l and MH at 450 µmol/L significantly increased the single fruit weight compared with the untreated control (Figure 5). Similarly, Ram et al. (2003) reported the increase in fruit weight of musk melon after GA_3 treatments.

Number of fruits/ plant

The lowest concentration of ethrel (700 µmol/L) and all concentrations of GA_3 and MH used resulted in significantly higher number of fruits/plant compared with the water soaked control (Figure 6). The results of water soaked control were statistically at par with that of untreated control. Comparing with untreated control, all growth regulator treatments significantly increased the number of fruits/plant. The highest number of fruits (25.7) was recorded with the application of MH at 450 µmol/L whereas, the minimum number of fruits/plant (4.9) was recorded in the untreated control. Endogenous IAA level which was greater in these treatments at

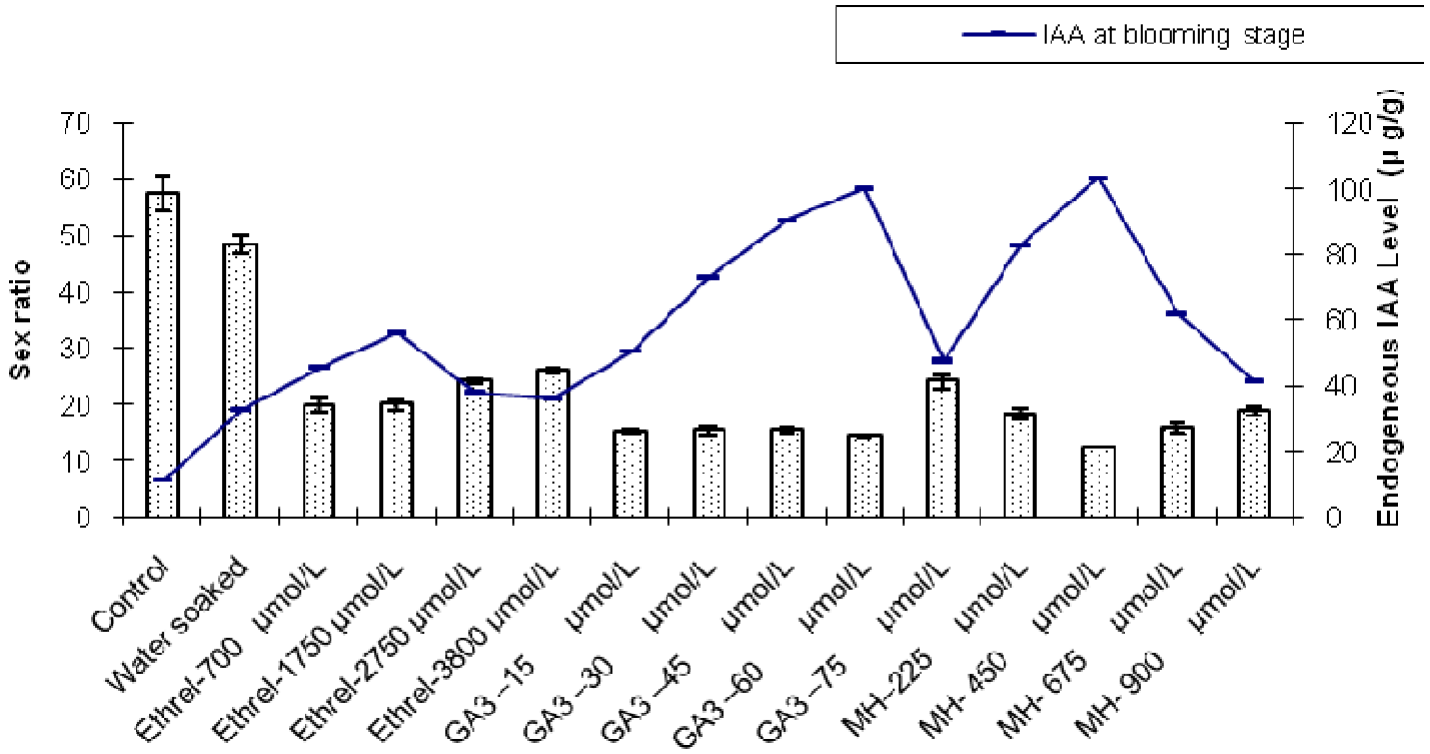

Figure 3. Effect of plant growth regulators on sex ratio (means of two years with 3 replications per year) and its interaction with endogenous IAA level at blooming stage. Vertical bars show the standard error means.

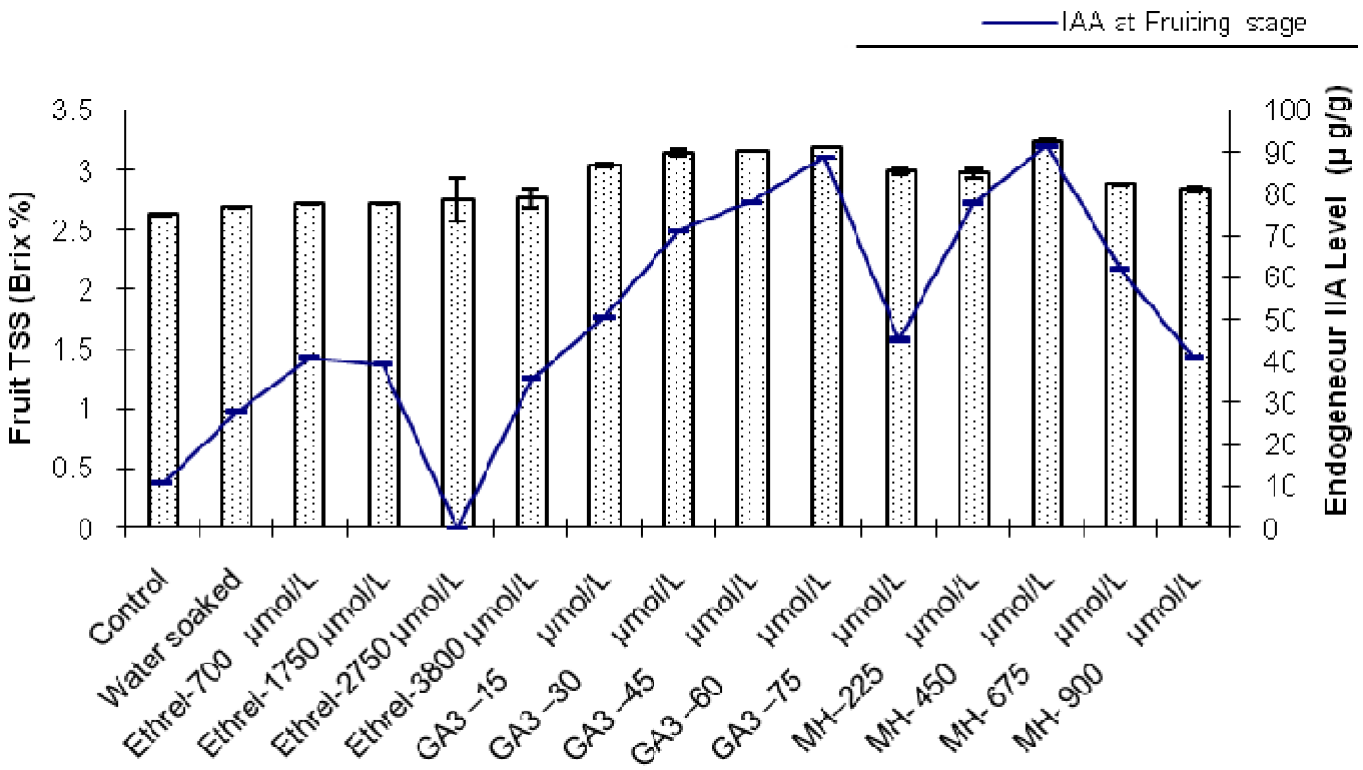

Figure 4. Effect of plant growth regulators on TSS (Brix %) (means of two years with 3 replications per year) and its interaction with endogenous IAA level at blooming stage.Vertical bars show the standard error means.

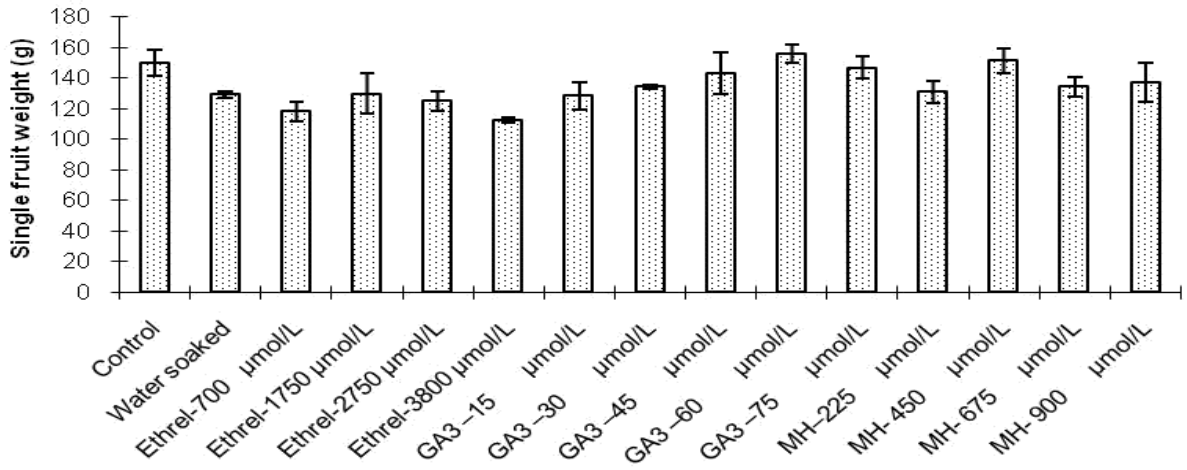

Figure 5. Effect of plant growth regulators on single fruit weight (g) (means of two years with 3 replications per year).Vertical bars show the standard error means.

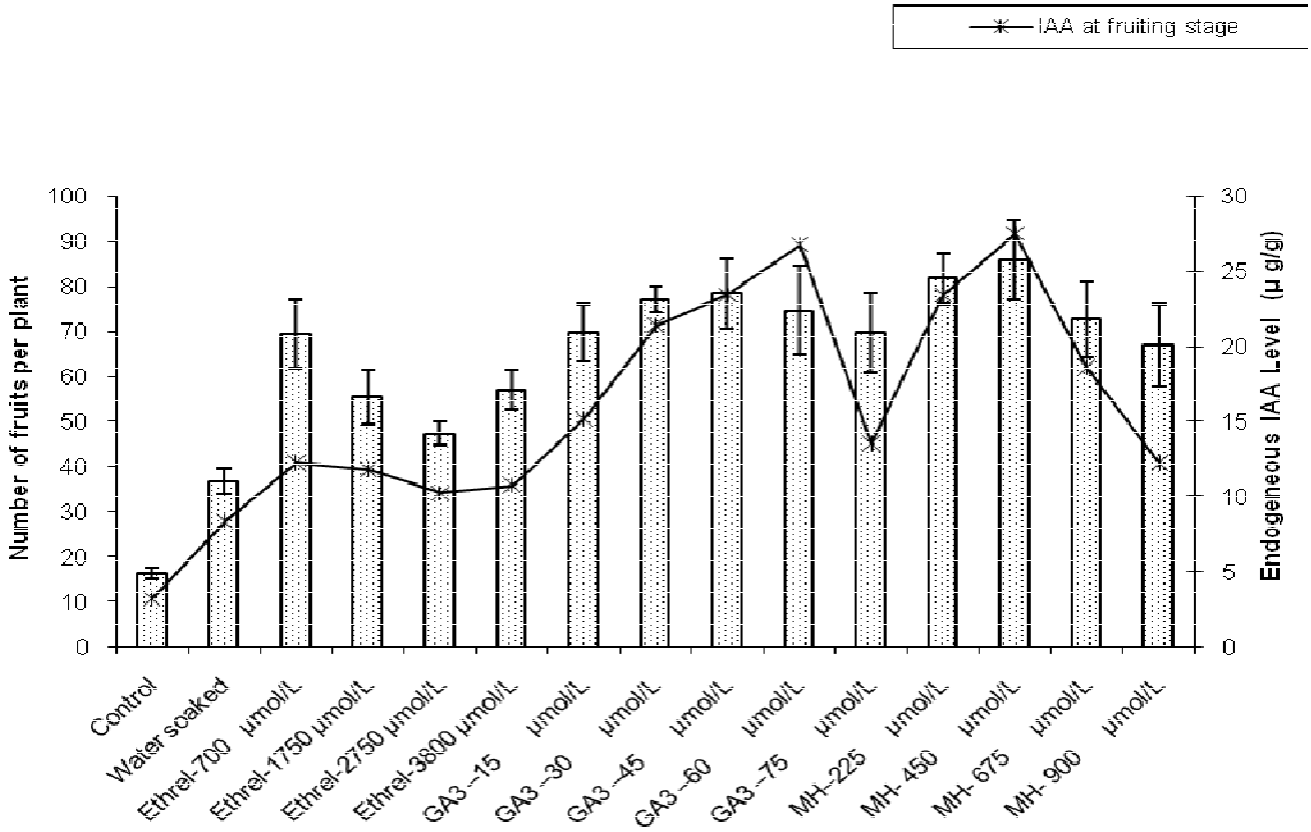

Figure 6. Effect of plant growth regulators on fruits per plant (means of two years with 3 replications per year) and its interaction with endogenous IAA level at fruiting stage. Vertical bars show the standard error means.

fruiting stage had a significant correlation with number of fruits/plant (r=0.666). The highest number of pistillate flower/ plant were recorded at MH at 450 µmol/L which had a significant positive correlation with number of fruits/plant (r=0.710) (Table 2). These results support the findings of Rafeekher (2002) and Kshirsagar (1995) who reported that MH, GA_3 and ethrel were effective in increasing number of fruits/plant in cucumber.

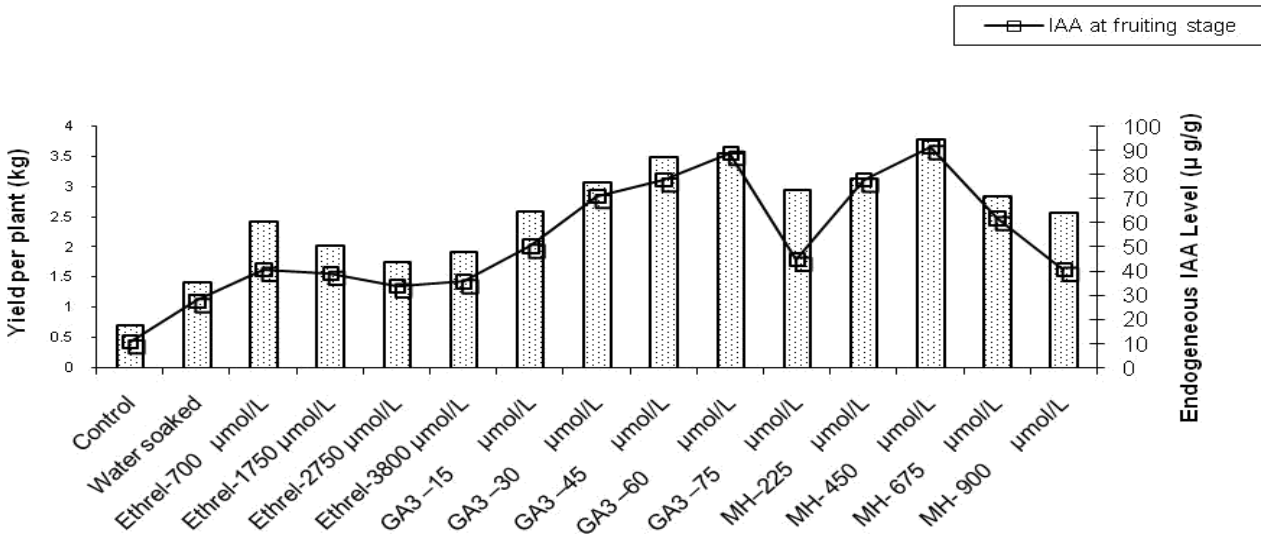

Figure 7. Effect of plant growth regulators on yield per plant (kg) (means of two years with 3 replications per year) and its interaction with endogenous IAA level at fruiting stage. Vertical bars show the standard error means.

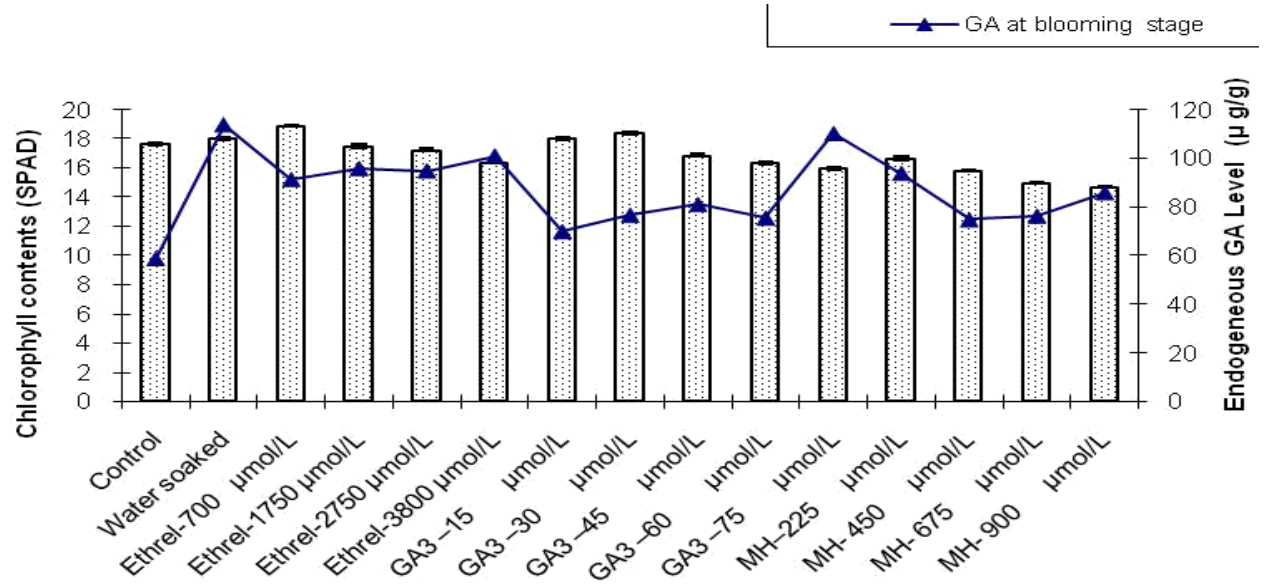

Figure 8. Effect of plant growth regulators on chlorophyll contents (SPAD) (means of two years with 3 replications per year) and its interaction with endogenous GA level at blooming stage. Vertical bars show the standard error means.

Fruit yield/ plant

Growth regulator treatments had a significant effect ($p \leq 0.01$) on the fruit yield/plant compared with control. Yield increase following the application of growth hormones ranging from 147 to 435% compared with untreated control. The maximum fruit yield/plant (3.80 kg) was recorded with MH at 450 µmol/L (Figure 7) followed by GA_3 at 60 µmol/L, respectively. Endogenous IAA level had a significant correlation with fruit yield/plant (r= 0.757) (Table 2). Many workers have reported similar results that GA_3 significantly increased yield in cucumber (Hussain et al., 1990).

Chlorophyll contents of leaves

Water soaking seed treatment increased the chlorophyll contents of the leaves as compared to untreated control (Figure 8). Low concentration of ethrel (700 µmol/L) and GA_3 at 30 µmol/L increased the chlorophyll contents of

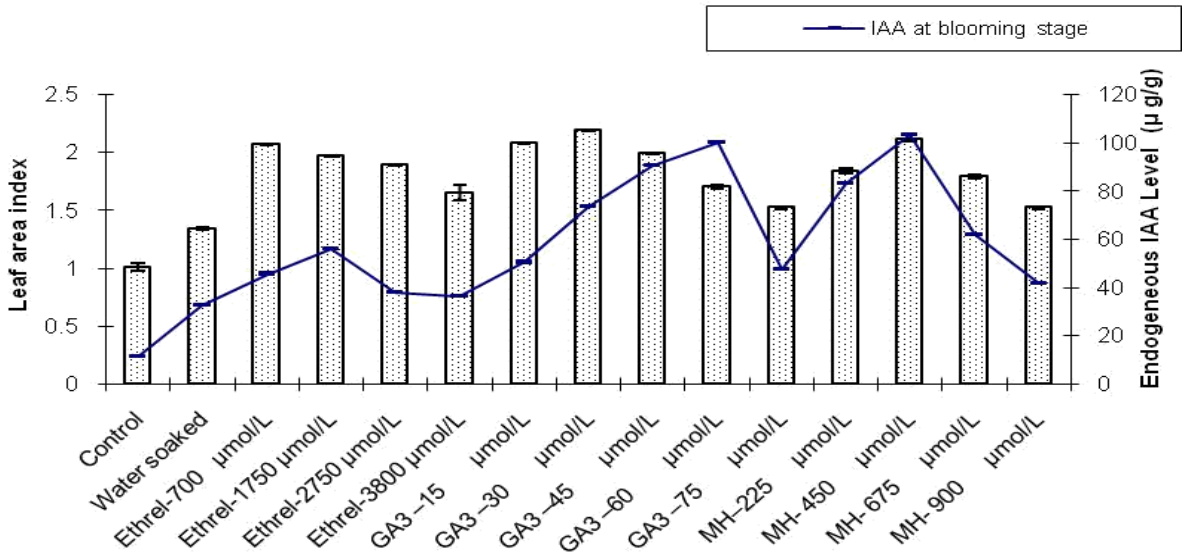

Figure 9. Effect of plant growth regulators on Leaf Area Index (LAI) (means of two years with 3 replications per year) and its interaction with endogenous IAA level at blooming stage. Vertical bars show the standard error means.

leaves while all other treatment decreased the chlorophyll contents compared to water soaked and untreated controls. Minimum level of chlorophyll contents was recorded with MH at 900 µmol/L.

Leaf area index

Soaking of seeds in water and growth regulators significantly increased the Leaf Area Index (LAI) as compared to untreated control (Figure 9). Maximum LAI was recorded with GA_3 at 30 µmol/L followed by MH at 450 µmol/L. However, minimum level of LAI was recorded in untreated control.

Endogenous hormone level

Although previous studies discuss the effects of growth hormones for sex expression in cucumber, they do not provide enough information on the putative role of the endogenous level of phytohormones. Our studies have clearly demonstrated that pre sowing seed treatment with all growth hormones resulted in the increase in endogenous GA_3 and IAA as compared with un-treated control (Table 1). The increase was relatively higher for IAA. Maximum GA_3 and IAA level was recorded at blooming stage after the treatments of GA_3 75 µmol/L and MH 450 µmol/l, respectively. IAA level had a significant negative correlation with sex ratio and positive significant correlation with the number of pistillate flowers/plant (Table 2). It is noteworthy that the seed soaking in aqueous solutions of growth hormones has resulted in marked increase in the GA and IAA contents in leaves as compared to un-soaked control. Different hydrolytic enzymes are activated on imbibitions releasing these growth promoting hormone in free form from the stored conjugated form. Ogawa et al. (2003) assessed correlations between GA biosynthesis and response by in situ hybridization analysis and demonstrated that the expression of GA-responsive genes is not restricted to the predicted site of GA biosynthesis, suggesting that GA itself, or GA signals, is transmitted across different cell types during Arabidopsis seed germination. Yamaguchi and Kamiya (2002) described that in dicots de novo GA biosynthesis after seed imbibitions is essential for seed germination.

At two leaf stage, seed soaking treatments increased GA_3 level compared to untreated control. However, the increase was more pronounced in MH treatments compared to GA_3 and ethrel. Maximum endogenous GA_3 level (55.5 µg/g) was recorded with MH at 225 µmol/L. At flower initiation stage also, the maximum endogenous GA_3 level (89.7 µg/g) was recorded with MH at 225 µmol/l. Except ethrel at 700 µmol/l, all growth regulator seed treatments increased the GA_3 level at flower initiation stage compared to 2 leaf stage and untreated control but the endogenous GA_3 level was high in water soaking treatment compared to ethrel treatments at flower initiation stage. At blooming stage, all treatments increased the GA_3 level over the flower initiation stage and untreated control. Maximum GA_3 level (113.7 µg/g) was recorded at water soaked control. During fruiting stage, maximum GA_3 level (48.3 µg/g) was recorded with MH at 675 µmol/L. In all treatments, endogenous GA_3 level increased from two leaf stage up to blooming stage but decreased at fruiting stage.

At two leaf stage, maximum level of endogenous IAA

was recorded with MH at 225 µmol/L. Seed soaking treatments increased IAA level at two leaf stage compared to untreated control though there was no marked difference between the water soaked and untreated control. Among ethrel treatments, the highest IAA level was recorded with low concentration (700 µmol/L), increase in the concentration decreased IAA production. At flower initiation stage, the endogenous IAA level decreased following application with GA_3 at 45 µmol/L and with higher concentrations (2750 and 3800 µmol/L) of ethrel. All other treatments increased IAA level compared to that of two leaf stage being maximum with MH at 225 µmol/L. IAA level decreased in water soaked treatment compared to untreated control. At blooming stage, as well as at fruiting stage increase in IAA level was at higher concentration of MH at 450 µmol/L followed by GA_3 at 60 µmol/L as compared to untreated and water soaked control.

GA_3 level increased from two leaf stage to fruiting stage even in unsoaked treatment whereas, IAA had no such age related effect and was higher at blooming stage in water soaked treatment similar to GA_3. Baydar and Olger (1998) reported that level of GA_3 in leaves increased during the bolting stage. Both GA_3 and IAA were less at fruiting stage. Yoruk et al. (2005) reported that the concentration of GA_3 increased during the period of rapid growth in April and May. The concentration of IAA decreased slightly from January until March, increased markedly in April and then decreased again after the cessation of rapid growth in *Rosa Canina*.

Conclusion

Seed soaking treatment with maleic hydrazide at 450 µmol/l led to the highest increase in the number of pistillate flowers/plant and lowest in sex ratio resulting in maximum number of fruits and fruit yield/plant. This may be attributed to its effect on increasing IAA level. There was a positive significant correlation between endogenous IAA level and pistillate flowers. Fruit yield also had a positive significant correlation with endogenous IAA level. In summary, MH at 450 µmol/L has shown to have better potential at commercial level as seed soaking.

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