THE EFFECT OF 2-(CHLOROETHYL) TRIMETHYLAMMONIUM CHLORIDE (CCC) AND GIBBERELLIC ACID ON THE ANATOMY OF *HELIANTHUS ANNUUS* GROWN AT DIFFERENT SOIL MOISTURE REGIMES

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Anatomical changes induced by 2-(chloroethyl) trimethylammonium chloride (CCC) and Gibberellic acid (GA) on *Helianthus annuus* grown at 100 and 30 per cent soil moisture regimes (SMR) were studied and discussed. It was concluded that CCC-treated plants produced anatomical changes which confer advantageous adaptations. The general anatomical studies of the stems of the CCC-treated plants showed an increased development of vascular tissues. Lowest cortex/stele ratio (1.7, 1.55) both at higher and lower SMRs was recorded for the stems of the CCC-treated plants as compared to the control (2.33, 2.18) and GA-treated (2.8, 2.69) respectively. Increase in the number of vessels, number of palisade layers and compactness of the cells was observed in the leaves of the CCC-treated plants. There was also an increase of vascular tissues as a result of which the cortex/stele ratio was also found to be lowest among the leaves of the CCC-treated plants. The area of the vascular region in the roots of the three sets of plants at 30 per cent was increased compared to that of the higher SMRs. In the CCC-treated plants the area of the vascular region of the roots at 30 per cent SMR was double that at the 100 per cent SMR.

INTRODUCTION

Little published information is available on the effect of CCC and GA on anatomical changes in plants. Mayr & Presoly (1963) studied the anatomical changes induced in wheat plants with the application of CCC and recorded an increase in the size of the hypoderm ring, parenchyma ring and number of vascular bundles. Stant (1963) with a number of experiments on *C. olitorius*, *H. cannabis* and *C. sativa* observed that GA accelerates and increases the longitudinal growth or extension of the cell. None of the studies described so far indicate the changes in anatomy of the CCC and GA-treated plants grown under moisture stress. Anatomical studies were, therefore, carried out to compare the anatomical changes of the normal (untreated), CCC-treated and GA-treated plants grown at decreasing moisture regimes. The first paper in this series (Baig, 1970) described the effects of CCC and GA on the growth of

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Helianthus annuus at 100, 55,30, 15 and 10 per cen tmoisture regimes. This paper presents the anatomical studies of the same plants (at final harvest) which were used for studies of growth.

METHODS

The effect of the decrease in the soil mositure regime, on the anatomical features of the three sets of plants was studied. The material used for section cutting was fixed from the same set of plants which were used for growth studies (Baig, 1970). Stems, leaves and roots, of comparable age and developmental stage were chosen and selected portions were fixed in formalin acetic alcohol and after dehydration and infiltration, the material was embedded in molten wax at 58°C. Transverse sections were then cut between 6-8 μ by means of the microtome. Because of the considerable time and labour involved in section cutting, studying and measuring the sections of the stems, leaves and roots of all three sets of plants, at five mositure regimes, it was thought that the change in anatomical features, as affected by the decrease in the soil moisture regime might be well represented by simply studying the anatomy of the plants belonging to the 100 and 30 per cent soil moisture regimes. These plants could indicate a general trend of change in the anatomical features when subjected to adverse water conditions.

The following features of the anatomy of the stems, leaves and roots were studied :

Stem. Transverse sections from the middle of the first internodes were chosen for studying the anatomy of the stems in all three sets of plants. The number of vascular bundles were counted. The outlines of xylem, phloem and sclerenchymatous fibres of five largest bundles were drawn using Camera lucida under low power. The area of these was recorded using a planimeter. The average area of xylem, phloem and sclerenchyma per vascular bundle was determined. The area of the cross section of stem was then determined under low power. The average areas of the xylem, phloem and sclerenchyma were then expressed as percentages of stem cross section area.

Leaf. The material for sections of leaves was taken from the centre of fully mature leaves in each case. The areas of the vascular tissues were determined in the same manner as for the stem. The number of vessels was counted, the diameter of vessles was measured. The area of cross section of the mid-rib was determined, the number of palisade layers and the degree of compactness of the spongy tissue was compared.

Roots. The area of the cross sections of the roots was determined. The area of the vascular tissues internal to the pericycle and the area of the cortex was determined in the same way as done for stems and leaves. These areas were expressed as percentage cross section of root.

The values of occular divisions under high power (x 40), low power (x 10) and differential objective (x 2) magnification were 3.7, 16.1 and 76.9 μ respectively. The magnification of Camera lucida drawing was 183 at low power (x 10) and 34 at (x 2).

EXPERIMENTAL RESULTS

The anatomical features studied in the stems of the controls, GA and CCC-treated plants at the 100 and 30 per cent soil moisture regimes are given in Table 1.

TABLE 1. *Anatomical Features of the Stem of Controls, GA and CCC-treated plants.*

Moisture Regime	No. of vascular bundles	Areas of xy. ph. & Scl. as % area of x section of stem		
		xylem cm^2	phloem cm^2	sclerenchyma cm^2
Control 100%	12	0.217	0.24	0.268
GA 100%	12	0.237	0.183	0.231
CCC 100%	12	0.42	0.333	0.381
Control 30%	12	0.282	0.322	0.336
GA 30%	12	0.259	0.233	0.357
CCC 30%	12	0.638	0.487	0.586

It was observed that with a decrease in the SMR, there was an increased development of the vascular tissues. The area of the xylem, phloem and sclerenchymatous fibres was greater in the stems of the 30 per cent SMR in the three sets of plants.

The increase of vascular tissues was more or less the same in the controls and GA-treated plants, but a tremendous increase in the bulk of xylem tissue of the CCC-treated plants at 30 per cent SMR was observed. The area of xylem was more or less double in the CCC plants than the areas of the controls and the GA-treated plants, at the 100 per cent soil moisture level. The phloem elements and the sclerenchymatous fibres also showed a better development in the CCC-treated plants at the 100 per cent moisture regime. The areas of the xylem, phloem and sclerenchymatous tissues of the CCC-treated plants at the 100 per cent SMR were also higher than those of the controls and the GA-treated plants and with decrease in the SMR the CCC-treated plants showed a marked increase in the development of the vascular tissues than the controls and the GA-treated. The number of fully developed vascular bundles was practically the same. However, in the CCC-treated plants the number of under-developed bundles was greater and the cells seemed to be smaller and more compact, i.e., with smaller intercellular spaces as compared to the controls and particularly to the GA-treated ones, where the cell size was bigger with larger intercellular spaces.

The anatomical features of the leaves of the controls, CCC & GA-treated plants are shown in Table 2. It was observed that the degree of compactness of the spongy tissue, the number of vessles, the number of palisade layers, the area of the vascular tissues was increased in the three sets of plants at the lower mositure regime compared with the 100 per cent treatment. The diameter and number of larger vessels at 30 per cent was slightly reduced in the controls and GA-treated plants but it increased in the CCC-treated ones. It was also observed that the leaf tissues were more compact, with greater number of palisade layers, larger number and greater diameter of vessels, and larger areas of vascular tissues in the CCC-treated plants. Thus CCC-treated plants showed a greater development of xeromorphic characters.

Table 3 shows the diameter of ten largest xylem vessels in the central bundle of the mid-rib of the leaves. At the 100 per cent moisture regime the diameter of vessels was greater among the GA-treated plants, intermediate among the controls and the smallest among the CCC-treated plants. It was interesting to note that at the 30 per cent SMR the diameter of vessels among the CCC-treated plants had increased whereas, in the controls and GA-treated it was slightly reduced.

The anatomy of the roots of the three sets of plants was studied by measuring the areas of the region internal to the pericycle, *i.e.*, the vascular region and the areas of the region external to the pericycle, the cortical region of the roots. This is given in Table 4 which revealed that the area of the vascular region in the roots of the three sets of plants was increased compared to that of the corresponding higher soil moisture regimes. At the 100 per cent moisture regime the areas of the vascular and cortical regions were more or less the same in the Controls and the Gibberllic treated plants. However, in the CCC-treated plants the area of the vascular region was slightly larger and that of the cortical region smaller. It can also be seen from the table that in the CCC-treated plants the area of the vascular region of the roots was much larger. For example at 30 per cent soil moisture regime it was more or less double than at the 100 per cent soil moisture regime.

In the Gibberellic treated plants the area of the vascular region at the lower moisture regime was increased by half only and the increase in the Controls was very small. At the 30 per cent soil moisture regime the cortex/vas. reg. ratio was also much smaller in CCC-treated roots as compared to the controls and Gibberellic treated ones.

TABLE 2. *Anatomical features of the Leaves of the Controls, Gibberellic and C.C.C. treated plants*

Plant Type	No. of vessels in central bundle	No. of Palisade layers	degree of compactness of spongy tissue	Areas of xy. ph. scl. as % area of x section of mid rib.			No. of vessels over 14μ in central bundle
				xylem cm^2	pholem cm^2	scleren-chyma cm^2	
Control 100%	29	1	not very compact	4.75	4.92	4.86	14
Gibberellic 100%	22	2	compact as compared to control	5.7	5.24	5.9	17
C.C.C. 100%	30	2	more compact than control and Gibb.	7.35	4.61	5.38	18
Control 30%	32	2	Compact than 100%	5.54	5.91	5.74	13
Gibberellic 30%	24	2	more compact than 100%	5.78	5.53	6.42	14
C.C.C. 30%	35	3	compact than 100%	11.1	6.66	8.0	20

TABLE 3. *Diameters of Ten largest vessels of the Controls, Gibberellic and C.C.C. treated plants*

CONTROL		GIBBERELLIC TREATED		C.C.C. TREATED	
100% vessel diam (μ)	30% vessel diam (μ)	100% vessel diam (μ)	30% vessel diam. (μ)	100% vessel diam. (μ)	30% vessel diam. (μ)
40.7×29.6	33.3×29.6	33.3×27.7	33.3×33.3	37.0×29.6	40.7×33.3
37.0×25.9	25.9×22.2	37.0×27.7	25.9×18.5	29.6×18.5	37.0×27.7
29.6×22.2	37.0×22.2	37.0×29.6	22.2×20.3	29.6×22.2	40.7×27.7
25.9×25.9	37.0×22.2	37.0×31.4	16.6×14.8	33.3×25.9	33.3×25.9
33.3×25.9	29.6×22.2	29.6×22.2	14.8×14.8	22.2×22.2	29.6×25.9
25.9×22.2	22.2×18.5	29.6×25.9	22.2×18.5	22.2×18.5	29.6×25.9
22.2×22.2	22.2×18.5	29.6×22.2	18.5×18.5	18.5×18.5	27.7×20.3
25.9×22.2	22.2×14.8	29.6×22.2	22.2×18.5	25.9×18.5	33.3×22.2
25.9×25.9	25.9×22.2	29.6×25.9	22.2×20.3	25.9×18.5	25.9×24.0
22.2×25.9	14.8×18.5	29.6×22.2	18.5×18.5	22.2×18.5	29.6×18.5

This showed that there was a large response to CCC-treatment in the roots of plants grown at the lower soil moisture regime. The cortex/vas. reg. ratio in in the Controls and the Gibberellic treated plants was also smaller but not so great a reduction as in the CCC-treated plants. This showed that although CCC treatment had the effect of producing a measure of "pre-adaptation" further adaptation was still possible at the lower mositure regime.

TABLE 4. *Anatomical Features of the Roots of Controls, Gibberellic and CCC treated Plants*

Moisture Regime		Areas of vas. & cort. regions as % area of x section of root		Ratio of cortex
		vascular region cm²	cortical region cm²	vas. reg.
Control	100%	20.5	79.4	3.87
Gibb.	100%	20.9	79.0	3.77
CCC	100%	21.6	78.3	3.6
Control	30%	23.0	76.9	3.33
Gibb.	30%	28.6	71.3	2.5
CCC	30%	41.6	58.3	1.4

The Table 5 shows the cortex/stele ratio expressed as percentage of the stem cross section area. It was observed that with a decrease in the SMR, the cortex/stele ratio became smaller indicating that at the lower moisture regime there was a greater production of stele, particularly among the CCC-treated plants.

TABLE 5. *The cortex/stele ratios of the stems of Controls, gibberellic and CCC treated plants*

Moisture Regime		area of cortex as percentage of stem x section area cm²	cortex/stele ratio
CT	100%	20.2	2.33
GA	100%	21.9	2.8
Control	100%	23.2	1.71
Control	30%	24.62	2.18
GA	30%	27.52	2.69
CCC	30%	32.0	1.55

Table 6 shows the area of the cortex expresed as a percentage of the mid-rib cross section area and the cortex/stele ratio of the three sets of plants. The area of the cortex was smaller in the CCC-treated plants as compared to the controls and GA-treated ones and the area occupied by the stele was also greater among the CCC-treated plants because of the smaller value of the cortex/stele ratio.

TABLE 6. *Cortex/stele ratios of the leaves of Controls, GA & CCC treated plants*

Moisture regime		area of cortex as percentage of mid rib × section area cm²	cortex/stele ratio
Control	100%	85.45	5.87
GA	100%	83.16	4.93
CCC	100%	82.63	4.69
Control	30%	82.8	4.77
GA	30%	82.25	4.63
CCC	30%	74.16	2.87

DISCUSSION

By studying the anatomy of the stems, leaves and the roots of the CCC-treated plants, GA-treated and the controls, it was concluded that as the moisture regime decreases the plants of all three sets tended to respond to adverse water conditions by developing xeromorphic characters. In particular this consisted of an increase of vascular tissues in the stems and roots, an increase in the leaf, increase of the number of vessels, number of palisade layers and greater compactness of the cells. All these can be considered advantageous in water relations of the individuals possessing them. The degree of xeromorphic characters developed, varies from species to species, some are more responsive to drought than others. The application of CCC induces the xeromorphic characters even under mesophytic conditions and increasingly so with more adverse water conditions. The treated plants, when grown under mesophytic condition are already "pre-adapted" to water stress and, therefore, have a greater chance of survival, should any period of sudden drought occur.

The general anatomy of stem of CCC and GA-treated plants as compared to the control showed important points of difference in the extent of development of mechanical tissues especially the xylem elements. The ratio between the xylem area of the CCC-treated plants and the control at 100 per cent SMR was 2 :1 whereas there was no marked difference between the GA-treated plants and the controls, indicating that GA as compared to CCC is not effective in bringing about useful anatomical changes. It was observed that the cortex/stele ratio at 100 and 30 per cent SMRs was also lowest (1.71, 1.55) among the CCC-treated plants, as compared to the control (2.33, 2.18) and GA-treated plants (2.8, 2.69).

The anatomical studies of leaf show that the CCC-treated plants as compared to control & GA-treated plants have increased vascularization due to which the cortex/stele ratio was lower. The number of palisade layers, degree of compactness of spongy tissue, number of xylem vessels and area of the vascular tissues was greater among the CCC-treated plants. The anatomy of the roots showed the same tendency to produce more vascular tissues in the CCC-treated plants which was indicated by the lowest value of the cortex/stele ratio (1.4) as compared to the controls (3.33) and the GA-treated roots (2.5).

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