Histological Reactions of Hemp Plant (*Cannabis sativa*) to Giberellic Acid

By

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The stimulatory influence of gibberellic acid on the activity of terminal meristems (Sachs & Lang 1957, Sachs, Bretz & Lang 1959, Sachs, Lang, Bretz & Roach 1960 and others) and of cambium (Bradley & Crane 1957, Wareing 1958) induced by gibberellin application points to a considerable mitotic activity of gibberellins. Not less noticeable, however, seems to be the ability of gibberellins to influence the direct differentiation of cambial products, the differentiation of single tissues and in this way to influence either directly or indirectly the whole anatomic structure and organigenesis of plants (Wareing 1958, Chailakhian, Kochankov & Zamota 1960, Razumov 1960, and others).

Having in mind the observations quoted, as well as our introductory studies (Herich 1960) in which after gibberellin application on seeds conspicuous changes in the anatomic structure of hemp stems were observed, we considered necessary a detailed investigation and mathematic-statistical rating of the changes brought about by gibberellin treatment.

Material and method

Our experimental work was carried out with hemp of the variety „Rastislavická". Material for study of the influence of gibberellic acid on the anatomic structure was represented by ♀ individuals taken from the lot of control plants and those the seeds of which had been allowed to soak 24 hours in 5 ppm concentration of gibberellic acid, whereafter the maximum of total stem elongation was registered. The plants were grown in soil, under normal field conditions. Stems of a total average length of 90—100 cms were analysed, 10 individuals from each alternative.

Total stem thickness, percentage of bark part, xylem, of parenchymous pith tissue and stem tube from the total cut were rated, as well as the percentage of primary and secondary bast fibres in the bark part, width and thickness of walls of single bast fibres. As the anatomic structure of stems in the various parts of stem is different, samples were taken from the lower, middle and top thirds of total stem length. When making microscopic preparations, the common microscopic technique was used. The cuts were stained with safranine and light green colour, chlor-zinc-iodine

and phloroglucine. The width of bast fibres and thickness of their walls were stated by help of an ocular micrometer; the percentage of the bark part, of xylem, parenchymous pith tissue and stem tube from the whole plain of cut were stated in the various thirds of total length as follows: the microscopic preparations were drawn on paper and from these drawings the percentage of the single parts were calculated. In the same way the percentage of primary and secondary fibres of bark part in the single stem thirds was obtained with the sole difference of drawing only the single field of view, not the whole bark part. The percentages given are the average of 20 fields of view.

The rating of results obtained was carried through by the method of analysis of normal variability. We express the possibility to prove difference by the probability "P" which was found for the relating figure "t" and for the given number of degrees of tolerance in Fischer's tables of probability.

Results

I. Influence of gibberellin treatment on histological structure of stems — on differentiation of xylem and bark

As I have already stated in the introductory part, the application of gibberellic acid to hemp seeds found its macroscopic expression as early as during growth by intensifying it, after the end of the vegetative period by elongating the total stem length.

The comparison of thickness of plants having an equal average length, taken from the control batch with those the seeds of which were treated with 5 ppm concentration of gibberellic acid, shows (Table 1) that after gibberellin treatment the stem thickness decreases along the total length of stem.

Table 1. Influence of gibberellic acid on stem thickness (in mm)

		Stem diameter ($\bar{x} \pm 3 \cdot s_{\bar{x}}$)	%%	t	P
Lower third	Control	3,75 ± 3 . 0,13	100,00	1,09	P=0,30
	Gibberellic acid	3,52 ± 3 . 0,17	93,86		
Middle third	Control	3,11 ± 3 . 0,13	100,00	1,30	0,30> P>0,20
	Gibberellic acid	3,05 ± 3 . 0,12	98,07		
Top third	Control	2,15 ± 3 . 0,23	100,00	0,35	0,80> P>0,70
	Gibberellic acid	2,04 ± 3 . 0,22	94,88		

$\bar{x}$ = average, $s_{\bar{x}}$ = median error of average, t = test of statistical significance, P = probability.

Table 2. Influence of gibberellic acid on anatomic structure of stems — percentage of bark, xylem, parenchymous pith tissue and stem tube from total plain of microscopic preparation

			$\bar{x} \pm 3 \cdot s_{\bar{x}}$	%%	P
Lower third	Bark	Control	15,61 ± 3 . 0,69	100,00	0,10> P>0,05
		Gibberellic acid	13,97 ± 3 . 0,44	89,49	
	Xylem	Control	57,49 ± 3 . 1,44	100,00	0,80> P>0,70
		Gibberellic acid	56,63 ± 3 . 1,76	98,50	
	Parenchymous pith tissue	Control	8,14 ± 3 . 0,48	100,00	0,90> P>0,80
		Gibberellic acid	7,95 ± 3 . 1,05	97,66	
	Stem tube	Control	18,73 ± 3 . 1,15	100,00	0,30> P>0,20
		Gibberellic acid	21,41 ± 3 . 2,02	114,30	
Middle third	Bark	Control	19,00 ± 3 . 1,01	100,00	0,10> P>0,05
		Gibberellic acid	16,63 ± 3 . 0,70	87,52	
	Xylem	Control	47,41 ± 3 . 1.18	100,00	P>0,001
		Gibberellic acid	37,40 ± 3 . 1,52	78,88	
	Parenchymous pith tissue	Control	13,90 ± 3 . 1,82	100,00	0,50> P>0,30
		Gibberellic acid	15,97 ± 3 . 1,61	114,89	
	Stem tube	Control	19,65 ± 3 . 2,82	100,00	0,02> P>0,01
		Gibberellic acid	29,96 ± 3 . 2,38	152,46	
Top third	Bark	Control	26,20 ± 3 . 1,37	100,00	0,20> P>0,10
		Gibberellic acid	23,51 ± 3 . 0,88	89,73	
	Xylem	Control	40,91 ± 3 . 1,28	100,00	0,02> P>0,01
		Gibberellic acid	35,93 ± 3 . 1,01	87,82	
	Parenchymous pith tissue	Control	22,89 ± 3 . 1,36	100,00	0,02> P>0,01
		Gibberellic acid	29,52 ± 3 . 1,92	128,96	
	Stem tube	Control	9,60 ± 3 . 2,20	100,00	0,70> P>0,50
		Gibberellic acid	11,00 ± 3 . 2,20	114,58	

The greatest decrease in thickness was observed in the basal third of stems where the thickness was reduced by 6,14% in comparison with control plants; in the middle third the difference of thickness decreases to 1,93%, in the top third stem thickness after gibberellin treatment again is reduced by 5,12% in comparison with control plants. The differences, however, are not in a single case statistically provable at a 5% rate of probability.

The changes in stem thickness induced by gibberellin application are accompanied by conspicuous changes in the anatomical structure of stems (Table No. 2).

When comparing the anatomic structure of stems in the basal part of control plants and of those from treated seeds it may be observed that after gibberellin treatment the percentage of bark is considerably reduced in this part of stem, the stem tube conspicuously enlarges at the same time. The percentage of xylem as well as that of parenchymous pith tissue in this part of stem also decreases, but the differences compared with control plants at a probability of 5% are not provable.

In the middle part of stems xylem is on the decrease to a conspicuous extent (by 21,12% compared with control plants) after treatment with gibberellic acid, the percentage of parenchymous pith tissue increases (by 14,89% compared with control plants) but the stem tube enlarges to a very considerable measure (by 52,46% compared with control plants). The percentage of bark in this part of stem shows a decrease (by 12,48%) in comparison with control plants, the difference at a 5% rate of probability, however, is at the limit of possibility to prove.

In the top third of stems xylem is noticeably retreating while parenchymous pith increases. The percentage of bark and of tube from the total plain of cut shows no provable differences in this part.

Rating the influence of gibberellic acid on the anatomical structure of hemp stems upon the whole, we may say that by gibberellin application an increased parenchymatization of stems accompanied by conspicuous decrease of xylem is inducted. As far as the bark part is concerned, after gibberellin treatment its percentage is reduced as well, mainly in the basal part, the reduction, however, is not so noticeable as with xylem, and as it is evident from the table, the differences are levelling up gradually towards the top so that in the upper third of stem they are no longer provable.

II. The influence of gibberellic acid treatment on differentiation of primary and secondary bast fibres

The changes in the anatomical structure of stems inducted by application of a 5 ppm concentration of gibberellic acid to seeds, found their expression also in the differentiation of bast fibres. The percentage af bast fibres from the plain of cut taken from the bark part is given in table Nr. 3.

Table 3. Influence of gibberellic acid on percentage of primary and secondary bast fibres.

			$\bar{x} \pm 3 . s_{\bar{x}}$	%%	t	P
Lower third	Primary bast fibres	Control	38,95 ± 3.1,77	100,00	0,60	0,20> P>0,10
		Gibberellic acid	40,57 ± 3.2,03	104,15		
	Secondary bast fibres	Control	3,84 ± 3.0,85	100,00	1,04	0,50> P>0,30
		Gibberellic acid	2,65 ± 3.0,76	69,01		
Middle third	Primary bast fibres	Control	40,64 ± 3.1,50	100,00	0,24	0,90> P>0,80
		Gibberellic acid	41,16 ± 3.1,54	101,27		
Top third	Primary bast fibres	Control	30,40 ± 3.1,14	100,00	0,80	0,50> P>0,30
		Gibberellic acid	32,09 ± 3.1,79	105,55		

As is evident from the table, the percentage of primary bast fibres increases after gibberellin treatment in all parts of stem length, whereas the percentage of secondary bast fibres is on the decrease.

When comparing the size of primary bast fibres (Table 4) with the percentage of their occurence in the single stem parts it appears that the increase of primary bast fibres in the lower and top thirds is accompanied by simultaneous enlargement of size of single primary bast fibres.

Table 4. Influence of gibberellic acid on size of primary bast fibres.

		Cell diametres (μ)	Thickness of cell wall (μ)			
			$\bar{x} \pm 3 . s_{\bar{x}}$	%%	t	P
Lower third	Control	29,4/19,3	8,33 ± 3 . 0,24	100,00	1,38	0,20> P>0,10
	Gibberellic acid	33,4/20,1	8,76 ± 3 . 0,23	105,16		
Middle third	Control	27,2/15,9	7,25 ± 3 . 0,15	100,00	2,71	0,01> P>0,001
	Gibberellic acid	27,5/14,5	6,68 ± 3 . 0,16	92,13		
Top third	Control	19,9/12,2	4,18 ± 3 . 0,14	100,00	0,11	P>0,90
	Gibberellic acid	23,7/11,5	4,16 ± 3 . 0,11	99,52		

Compared with this, in the middle third of stems where after gibberellin application the formation of primary bast fibres was not influenced substancially, the size of single primary bast fibres in comparison with control stems does not show noticeable changes either.

As is evident from the table, substantial changes take place in wall thickness of primary bast fibres.

The study of influence of gibberellin treatment on technological qualities of bast fibres will be the object of our further investigations.

Discussion

From the results of the studies quoted the inference may be drawn that by application of gibberellic acid to seeds substancial changes in the histological structure of hemp stems are being induced. Tough at present it is not yet possible to give a detailed explanation of the mechanism of gibberellin influence on differentiation of single tissues, it appears, however, that indirect activity is at work if we consider the quoted observations of WAREING 1958 and further physiological studies on mutual interrelations of the gibberellins and growth substances. We judge so from the range of observations (ASPREY, BENSON-EVANS & LYON 1958, BRIAN & HEMMING 1958, KUSE 1958 and others) according to which the gibberellins show biological activity only in presence of native auxin or synthetic growth promoting substances of the auxin group. It is presumed that when induction of shooting takes place, the gibberellins at the same time neutralize the systems inactivating indole-acetic acid, in consequence of which, at least in this first phase the gibberellins raise the level of growth substances.

WAREINGS 1958 studies on the interaction between indole-acetic and gibberellic acids also point out the close relation between gibberellic acid and growth substances in the organogenesis. He could observe maximum stimulation of xylem differentiation at simultaneous application of gibberellic and indole-acetic acids, whereas when applying gibberellic acid in the absence of exogenous indole-acetic acid he says that gibberellin "can stimulate division of the cambium, but that the derived cells undergo little vacuolation or lignification, so that typical xylem is not formed".

At present the process itself of xylem differentiation into its single components as well as the differentiation of the other cambial products is not cleared up enough as to its cause. It is known indeed that some biologically active substances (including native and synthetic growth promoting substances) such as auxin (JACOBS 1952, 1956), 2,4 dichlorophenoxyacetic acid (KIERMAYER 1958, STRUCKMEYER 1951), α-naphtaleneacetamide (MITCHELL 1940, STRUCKMEYER 1951), 2, 3, 5 tri-iodobenzoic acid (KIERMAYER 1958) etc., may influence xylem differentiation, induct increased or decreased lignification and thickness of cell walls, but the mechanism of their influence exercised upon the cambium, its ability to differentiate are not sufficiently explained by these studies. It seems that here a complete system of "special forming reactions" is at work, as DOSTAL 1959 presumes. Attention should be drawn to KIERMAYERS 1958 supposition that "for development of thinwalled parenchymous cells probably higher contents of growth substances, for development of thickened sclerenchymous cells inhibitory substances (overdosed growth promoting substances) must be present".

When comparing the dynamic of primary and secondary xylem differentiation in the course of ontogenetic plant development which is followed up in a range of studies, two systems may be observed: 1. The system which conditions the primary (spring) xylem that, in turn, is conditioned by (a) the influx of heteroauxins from burgeoning buds, young leaves as well as of heteroauxin made free during the process of metabolism in active cambium, (b) by the unknown factor X coming up from the roots. — 2. The inhibitory systems produced by adult leaves inducting differentiation of secondary tissues with considerably thick vascular walls and wood fibre walls. According to DOSTAL 1959 by these inhibitions also all growth is withheld with the exception of embryonal differentiation of winter buds which is made possible by the bud scales. Further cambial activity can be resumed only after loss of leaves.

Considering what has been said here, as well as the results of our studies we suppose that one of the possible explanations of the mechanism of gibberellin influence on differentiation processes of single tissues is the direct regulation of the level of growth promoting substances and growth-inhibiting substances which are taking part in the growth process as well as in the processes of differentiation. Increased parenchymatization, decreased lignification as well as decreasing percentage of xylem and secondary bast fibres inducted by gibberellin application bear witness of the fact that gibberellin treatment suppresses formation of growth-inhibiting substances which set limits to the growth processes and, in the sense of KIERMAYERS supposition, probably also to the differentiation of secondary tissues. The observed intensified growth processes as well as the changes in the histological structure corroborate the assumption that suppression of the inhibitory system induced by gibberellin treatment was, on the other hand, accompanied by a raising level of growth promoting substances. Definitive inferences will, of course, demande a range of further investigations.

Finally, we wish to express our thanks to Messrs. Kyova Fermentation Industries, Tokyo, who had sent us their highly effective gibberellic acid which was used in our experiments.

Summary

The paper discusses the histological reactions of hemp stems to treatment with gibberellic acid.

Comparing studies were carried out with ♀ individuals of equal total length (90—100 cms) taken from a batch of control plants and such the seeds of which had been 24 hrs soaked in 5 ppm concentration of gibberellic acid. Sowing of both kinds of seeds was made into soil under natural field conditions at the usual agrotechnical care.

When comparing the anatomical structure of stems it could be observed that after gibberellin application

(a) stem thickness decreases (at an equal total length),
(b) percentage of xylem as well as lignification of secondary xylem upon the whole is on the decrease,
(c) increased differentiation of parenchymous pith tissue is inducted (to the detriment of xylem) and stem tube enlarges to a substantial extent,
(d) in the lower part of stems percentage of bark is being reduced, the differences, however, are gradually levelling up,
(e) on grounds of anatomic rating of bark part percentage and size of primary bast fibres increase mainly in the basal and top thirds of total stem length, percentage of secondary bast fibres decreases, substantial changes take place in wall thickness of primary bast fibres.

It is presumed that by gibberellin treatment is brought about a suppression of inhibitory systems which are limiting growth processes and differentiation of secondary tissues, accompanied on the other hand by raising the level of growth promoting substances expressed in growth processes as well as in the anatomical structure upon the whole. The discussion deals with the relation between gibberellic acid and growth promoting substances to the differentiation of single tissues.

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