

STUDY OF REGENERATION IN PEARL BEAN AND SUNFLOWER SEEDLINGS

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Abstract

In experiments on root regeneration a study was made of the cell division, the variation in the amount of soluble protein, and the effects of β -indolylacetic acid and kinetin compared to the control.

In the course of the regeneration following the cutting-back of the rootlets of seedlings (Pearl bean, Iregi csikos sunflower) the amounts of soluble protein in the roots and shoots were higher than in the control, while the intensity of the cell division also developed similarly in the root meristem cells. Auxin and kinetin increased the amount of soluble protein in Pearl bean seedlings, and gave rise to inhibition in this period of the regeneration in sunflower seedlings.

Various inhibitors of protein synthesis also act on the division and elongation of the root cells (IVANOV, 1970). As a result of auxin treatment the root formation is accelerated and the elongation growth too is enhanced (DUBOUCHET, 1968; FELLEBERG, 1969). Indoleacetic acid treatment first increases, then decreases the growth, and later a second increase of rate ensues (TRUELSEN, 1966). Gibberellic acid inhibits regeneration (PREVOT, 1968). The regeneration of decapitated stem is inhibited by higher concentrations of kinetin (HILLMAN, 1970).

In our experiments on root regeneration a study has been made of the cell division, the variations in the amounts of soluble protein, and the effects of β -indolylacetic acid and kinetin compared to the control.

Materials and Methods

Experiments were carried out with white Pearl bean (Baranya county variety) and sunflower (Iregi csikos). Germination was performed on filter paper moistened with distilled water, in Petri dishes in a 23 °C thermostat. The reserve nutriment was used up for the growth of the seedlings and there was little nitrogen uptake from the once-distilled Szeged water.

In the first part of the experiments some of the 3-day seedlings were left as control, while the others were cut back to 1-3 of the rootlets. Regeneration of the rootlets began on the third day after the cutting-off. A study was made of the chromosomes and the development of the cell size in the meristem tissues of the regenerated and the control root apices by carmineacetic acid staining on fresh preparations. Subsequently the regenerated roots were again cut back and the division of the meristem cells of the root apices regenerated for a second time was compared with that for the once regenerated case and for the control.

In the second part of the experiments the variation of the total amount of soluble protein in the roots and shoots of the regenerated and control seedlings was examined as a function of the regeneration time. Some of the cut-back rootlets were treated with auxin, and others with kinetin. The two hormones were applied in a concentration of 1 mg/1000 ml deionized water. The total soluble protein was determined on the basis of the method of LOWRY et al. (1951).

Results and discussion

These experiments permit the conclusion that the regeneration initiates active processes of synthesis in the plants, thereby increasing the extent of the mitotic cell division and the growth associated with it. These results are summarized in Table 1.

Table 1. Variations in division and size of meristem cells in regenerated and control rootlets Sunflower Pearl bean

Variants	Rege- nera- tion time days	Once regenerated						Twice regenerated						Size of R cells as percentage of size of C cells	
		No. of cells studied		No. of cells dividing		Extent of division, %		No. of cells studied		No. of cells dividing		Extent of division, %		Length	Cross- section
		C	R	C	R	C	R	C	R	C	R	C	R		
Pearl bean	3	106	82	35	2	33.01	2.43	58	99	16	5	27.00	5.05	1.28	0.92
	5	56	192	18	66	32.14	36.26	72	40	21	25	29.16	62.50	1.25	0.60
	6	142	96	39	37	27.46	21.12	63	74	38	35	60.31	47.29	1.25	0.76
Sun- flower	3	188	175	103	32	54.78	18.20	153	238	73	187	47.70	51.80	0.71	0.63
	5	176	179	113	174	64.20	98.86	137	162	22	79	16.05	48.70	0.98	0.82
	6	198	157	161	114	81.31	72.61	50	22	7	2	14.00	9.00	0.10	0.90

Explanation of symbols: C=control
R = regenerated

It can be seen from the Table that the intensity of division of the regenerating rootlets exceeds that of the controls. The extent of division decreases with the progressing of the regeneration. The extent of the decrease may be brought about by the fact that there is a reduction of the synthesis processes, since the organ is regenerated and there is not sufficient nutriment for growth, and the elongation of the cells assumes prominence. The twice cut-back and again regenerating rootlets exhibit a more intensive division, but for a shorter time than when first regenerated. The

Table 2. Variation of the total amount of soluble protein in the rootlets and shoots as a function of the regeneration time. Calculation: mg/g fresh weight

Variants	Time from regenera- tion, days	Control		Regenerated		Auxin-treated		Kinetin-treated	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Sunflower	4	20.70	41.50	20.70	36.05	12.90	39.50	11.40	39.60
	5	13.80	35.55	22.20	36.75	17.30	40.90	18.50	39.80
	6	12.80	36.75	17.85	42.75	18.00	39.60	17.10	40.00
	7	14.50	25.50	18.50	33.45	18.80	27.50	17.90	33.90
	8	15.00	19.35	16.50	26.40	16.40	17.60	17.30	17.80
Pearl bean	4	19.50	40.35	23.55	33.60	22.65	39.15	25.35	31.80
	5	19.80	32.40	27.45	32.70	24.30	40.35	22.35	26.26
	6	17.55	32.70	17.85	24.90	23.85	35.20	18.15	24.15
	7	16.00	17.10	17.70	26.25	16.80	31.80	17.40	20.85
	8	15.15	18.45	15.75	16.80	18.60	33.15	18.30	12.00

size of the cells too is decreased in comparison to the control. In the second regeneration there is not a sufficient supply of ions to induce the turgor, and hence the elongation of the cell, which would be enlarged, does not take place. The minimum nutrient supply no longer exists, and accordingly the optimum plasm consistency of the cells can not develop.

Table 2 shows the change in the total amount of soluble protein in the rootlets and shoots of the regenerated and the control seedlings. The effects of auxin and kinetin on the regeneration are also presented here.

It can be seen that the total amount of soluble protein is greater in the regenerated roots and shoots, for because of the reformation of the organ the synthesis of proteins is more intensive than in the controls. The effects of auxin and kinetin on the regeneration are affected by the species, age and mode of treatment of the plant. This was also found in our experimental plants too. The two hormones affected the regeneration of the two plants in different ways. Our data show that both auxin and kinetin accelerated the regeneration in the rootlets of the Pearl bean, and stimulated the protein synthesis, for the amounts of soluble protein in the roots and the shoots are high. The concentration of kinetin employed inhibited the growth of the shoots of Pearl bean, and the amount of protein examined was concentrated in a smaller part.

In sunflower seedlings auxin and kinetin periodically inhibit and stimulate the regeneration of the rootlets, depending on the age of the seedlings. If the total amount of soluble protein rises above the value for the control in the course of the regeneration, both hormones act as inhibitors, whereas in other cases they give rise to stimulatory effects.

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