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EFFECT OF PHOTOPERIOD, SEED VERNALIZATION AND GROWTH REGULATORS ON GROWTH, DEVELOPMENT AND ENDOGENOUS HORMONES IN CARROT *VAR.* NANTES

RAM CHANDRA HEBBAR[1], G.S.R. MURTI, K.K. UPRETI AND M. UDAYKUMAR[2]

Plant Hormones Laboratory
Division of Plant Physiology and Biochemistry
Indian Institute of Horticultural Reserach
Hessaraghatta Lake Post, Bangalore 560 089

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SUMMARY

The investigations reveal that the carrot var. Nantes responds to photoperiod. LD enhanced morphological characters and promoted flowering. Vernalization of seeds was ineffective in altering the growth and development of carrot plants. GA_3 at 100 mg/l as seed soaking treatment did not influence either growth parameters or flowering. The growth promoters, cytokinins were higher in plant exposed to LD. Z/DHZ was predominant cytokinin observed under long photoperiod in carrot. The growth inhibitor, ABA was markedly higher under SD in plants raised from both unvernalized and vernalized seed. The growth and development of carrot plant is discussed in relation to the environmental variables and endogenous hormones.

INTRODUCTION

Carrot is a cold requiring biennial responsive to long photoperiods in flowering (Fisher, 1956; Atherton *et al.*, 1984). Normally the carrot root is vernalized for the promotion of flowering. Seed vernalization is effective in few crops like radish (Yoo and Uemoto, 1976). Information on the efficacy or otherwise of seed vernalization on flowering and root development in carrot is scanty. Further it was of interest to explore the possibility of using growth hormone like gibberellic acid (GA) substituting its cold requirement, if any for flowering.

Photoperiod influences the development of storage organs such as tubers in potato (Garner and Allard, 1923a) and bulb in onion (Steer, 1980). The effects of photoperiods on root development in carrot, however, have not received the due attention. The foliar application of GA substitutes the photoperiodic (Schwab and Neumann, 1975) and chilling (Globerson, 1972) requirements for flowering in carrot. However, the effectiveness of GA as seed soaking treatment on growth and development of carrot plants under contrasting photoperiods has not been studied. Although, carrot responds in flowering to photoperiod and vernalization, our knowledge of changes in endogenous hormones which acts as a chemical messenger during plant growth and development and relationship to the environmental variables is only fragmentary and inadequate. Hence. experiments were planted for studying the role of photoperiod on root development, the impact of seed vernalization on root development and flowering, the effectiveness of GA in substituting for the chilling requirement, and the changes in endogenous hormones, cytokinins and abscisic acid (ABA) in relation to photoperiod/seed vernalization.

MATERIALS AND METHODS

Carrot variety Nantes was used in all the investigations.

Vernalization of carrot seeds: The moisture content of the seeds was kept at 45-50 per cent by allowing them to imbibe water. The seeds were then transferred to a

1. Div. of Agriculture & Soil, NRSA, Dept. of Space, Balanager, Hyderabad-37.
2. Dept. of Crop Physiology, U.A.S., G.K.V.K., Bangalore-65.

perforated polythene bag and maintained at 6 ± 2 °C temperature for 42 days. At regular intervals, the moisture content of the seed was monitored. Another lot of seed was kept at room temperature to be used as unvernalized seed. The average maximum and minimum temperatures during this period ranged from 26.1-27.8 °C and 11.8-15.5 °C, respectively.

GA treatment of seeds: About 25g each of vernalized and unvernalized seeds were soaked for 15 hr in 200ml of GA (100 mg/l) solution, air dried and were sown in pots. The seeds in control were soaked in water for the same duration.

Photoperiodic treatments: Short days (SD: 8hr light + 16hr dark) consisted of light from 8.30 a.m. to 4.30 p.m. and dark for the rest of the period by cutting off the light using thick black cloth cover over a rectangular frame. Long days (LD: 16hr light + 8hr dark) were given by exposing the plants to natural sunlight during the day time and extending the light through artificial illumination by fluorescent tubes and incadescent bulbs in the night so as to obtain a total of 16hr continuous light. The illumination at the plant level during extended light period was about 100 foot candles. Carrot seeds, vernalized as well as nonvernalized, treated with GA, were used for raising plant material under both SD and LD in 20cm diameter earthen pots filled with soil and FYM (1:1). After the first sampling for the analysis of plant hormones, three plants per pot were retained. Observations on morphological characters, flowering and root shoot dry weights were recorded at 25, 50, 75 and 100 days. For the sake of brevity, the data on above parameters are presented for 100 days only.

Analysis of plant hormones: The analysis of plant hormones, ABA and cytokinins, was carried out at two growth stages: i.e. 30 days after sowing coinciding with the carrot root initiation, and 60 days after sowing coinciding with the active growth of carrot root.

Cytokinins were analyzed following the procedure described by Raja Rao *et. al.* (1983). 50g of tissue was macerated with 80 per cent methanol in a Waring blender and the tissue was re-extracted thrice, each for 24 hr at 3-5 °C with fresh solvent. The extract was brought to the aqueous phase *in vacuo* at 35 °C, adjusted to pH 3.0 and partitioned against equal volumes of diethyl ether. The aqueous fraction was adjusted to pH 8.0 and partitioned thrice against equal volumes of water saturated *n*-butanol. The butanol-soluble fraction was purified on ion-exchange column as described by Letham (1966 a, b). The separation of butanol-soluble cytokinins was carried out on Sephadex LH-20 column (Armstrong *et. al.*, 1969) using 35 per cent aqueous methanol. The cytokinin activity was detected by the soybean hypocotyl test (Newton *et. al.*, 1980) using the cv. Acme. The extract equivalent to 25g fresh weight of tissue was bioassayed. The number of explants per flask was three. The cytokinin activity was expressed as mean fresh weight of hypocotyl sections.

ABA was analyzed following the procedure followed by Saunders (1978) as modified by Murti (1988). 5g of the tissue was homogenized in 100ml of 80 per cent alkaline methanol. Extraction was done once again after 24 hr with fresh solvent. The combined methanolic extract was filtered and the extract was brought to aqueous phase *in vacuo* in a flash evaporator at 35 °C. The aqueous extract was partitioned against diethyl ether after pH adjustment to 3.0. This was followed by partitioning of the etheral extract against 5 percent sodium bicarbonate solution. The bicarbonate extract was then partitioned against diethyl ether at pH 3.0. The ether extract was then dried over anhydrous sodium sulphate, filtered and solvent evaporated. The ether soluble fraction was purified on an insoluble - polyvinyl pyrrolidone column. Further purification was carried out on HPLC (Waters Associates, Model 244) using μ bondapak column and an isocratic elution system of 40 per cent methanol at a flow rate of 1.0 ml/min. The quantification of ABA in HPLC purified fractions was performed on GLC (Hewlett Packard, Model 5840A) equipped with electron capture detector after methylation with etheral diazomethane. The column employed was 2.0m x 0.2cm. 2.0 per cent EPON 1001 column with nitrogen as carrier gas at a flow rate of 80ml/min. The temperatures of column, injection port and detector were maintained at 200, 250 and 300 °C, respectively. The quantification was done using an external standard of ABA.

RESULTS AND DISCUSSION

Observations on morphological characters revealed that the long photoperiod enhanced the plant height, leaf number, root length and its circumference, and shoot and root dry weights at 100 days (Table I). Seed vernalization and seed soaking treatment with GA showed no signifi-

Table I : Effect of photoperiod, seed vernalization and gibberellic acid on vegetative growth and dry matter accumulation in carrot plant (after 100 days)

Treatment	Plant height (cm)	Leaf number	Shoot dry wt. (g/plant)	Root length (cm)	Root circum-ference (cm)	Root dry wt. (g/plant)
SD.UnV.C	26.7	6.0	3.8	13.8	5.1	4.5
SD.UnV.GA	25.7	6.0	3.6	14.0	5.0	4.3
SD.V.C.	26.4	6.6	3.3	13.6	5.1	4.2
SD.V.GA	26.5	6.2	3.4	13.4	5.6	4.2
LD.UnV.C	29.8	7.0	6.3	15.6	9.6	9.6
LD.UnV.GA	20.1	8.0	6.5	16.4	9.2	10.2
LD.V.C	30.2	8.2	6.4	16.0	9.6	10.0
LD.V.GA	30.2	7.6	6.8	15.8	9.3	10.6

CD at 5% for V, GA, PxV, PxGA, VxGA and PxVxGA are non-significant

C-Control; SD- Short days; LD- Long days; V- Vernalized; UnV- Unvernalized
GA- Gibberellic acid; P- Photoperiod

Table II : Effect of photoperiod, seed vernalization and gibberellic acid on bolting and flowering of carrot plants

Treatment	Bolting		Flowering	
	No of plants bolting	Average days for bolting	No of plants flowering	Average days for flowering
SD.UnV.C	-	-	-	-
SD.UnV.GA	-	-	-	-
SD.V.C	-	-	-	-
SD.V.GA	-	-	-	-
LD.UnV.C	2.0 (13.6)	86.0	2.0 (13.6)	110.0
LD.UnV.GA	3.0 (21.7)	91.0	3.0 (21.7)	120.0
LD.V.C	3.0 (21.7)	92.0	3.0 (21.7)	115.0
LD.V.GA	4.0 (29.0)	90.0	4.0 (29.0)	113.0

Figures in the parentheses are percentages

Abbreviations—as given in table I.

cant influence on any of the above morphological characters. Interaction effects of photoperiod, seed vernalization and GA treatment of seed were not significant on morphological characters. Similar observations were made by Soffe *et. al.* (1977) in carrot and by Combe *et. al.* (1988) in radish. The increase in the above parameters under LD, however, could be due to an enhanced supply of photosynthates as a result of increased photosynthesis during prolonged exposure to sun light under LD. Garner and Allard (1923b) and Kellerman (1926) reported that carrot responded to LD in vegetative development, not forming an enlarged fleshy roots under SD. Seed vernalization and seed treatment with GA at the concentration tried were ineffective in altering the morphology of carrot plants. The information on the effects of seed vernalization and seed soaking treatments on carrot plant development is very scanty.

There was no bolting and flowering under SD (Table II). In this crop, the flowering behavior was erratic with only 13-30 per cent of the plants showing bolting and flowering under LD condition. There was wide variation in the number of days for flowering among the plants in any one treatment with no consistency in the number of days taken for bolting and flowering either with respect to vernalization or seed treatment with GA under LD. Promotion of flowering in carrot by LD has been reported by many workers (Lang, 1957 and Atherton *et. al.*, 1984). Contrary to the observations made by Hiler and Kelly (1985) the results from these experiments show that the LD are essential for flowering in carrot with no flowering noticed under SD. The results of the present study also reveal that seed vernalization and seed treatment with GA are ineffective in promotion of flowering. This is in contrast to the effects of seed vernalization on flowering in radish (Yoo and Uemoto, 1976). From the limited information available on vernalization of imbibed or germinating seeds, these results are only in a small percentage of flowering plants and thus are not a successful method of seed production (Hiller and Kelly, 1985).

Cytokinin activity: The cytokinin activity in the soybean hypocotyl test after sephadex LH-20 column chromatography was observed at elution volumes, 440-560 and 600-680ml (Fig. 1). There was one significant peak (440-560ml) under SD at both the stages of plant growth (30 and 60 days). Under LD, however, the peak at 440-560ml was significant only at 60 days while the peak at 600-680ml elution volume was prominent at both 30 and 60 days (Fig. 1). The elution volumes 440-560 and 600-680ml corresponded to those of authentic ZR/DHZR and Z/DHZ, respectively. Thus, the cytokinin-like substances ZR/DHZR were prominent under SD at both the stages whereas it was more marked at 60 days under LD. In contrast to this, the cytokinin activity of the substances

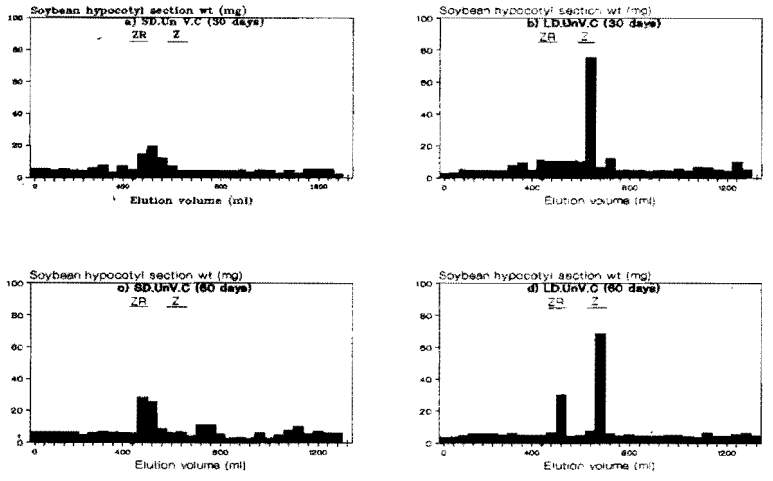

Fig. 1. Cell division activity in soybean hypocotyl test at 30 and 60 days of carrot plants exposed to two photoperiods.

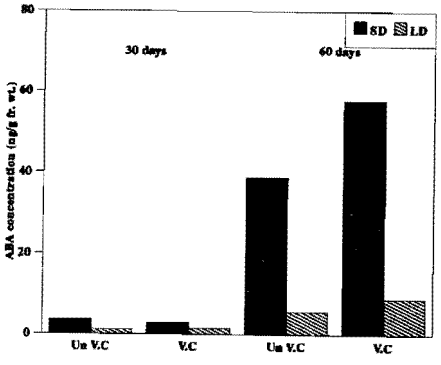

Fig. 2. ABA levels in carrot plants in relation to photoperiod and vernalization.

behaving like Z/DHZ were more marked under LD at both the stages. The total cytokinin activity was also higher under LD as compared to SD. These findings are in agreement with the fact that LDs promote cytokinin activity in several plants like Xanthium (Staden and Wareing, 1972) and onion (Lercari and Micheli, 1981).

ABA concentration: The ABA content at 30 days of plant growth was low and the differences due to photoperiod and vernalization were small (Fig. 2). The differences in ABA levels in relation to photoperiod were marked at 60 days of plant growth with plants under SD showing higher levels than those under LD. The changes in ABA due to seed vernalization were very small (Fig. 2). Similar observations under SD with respect to ABA were made by Moon and Lee (1986) in garlic and by Eagles and Wareing (1964) in sycamore.

It is thus seen from the results presented that there is an increase in cytokinin activity in carrot at both 30 and 60 days of growth under LD. Also, the concentration of the substance behaving like Z/DHZ in sephadex LH-20 column chromatography was remarkedly higher at both the stage under LD. It is believed that the over all growth of a plant is a response to the changes in relative concentrations of growth promoters and inhibitors. It is possible that there is a general increase in the activity of growth promoters and decline in the growth inhibitors under LD reflecting in an enhanced growth of carrot plants under this photoperiod as compared to SD. It is interesting to note that the levels of ABA in carrot plants grown under LD were lower as compared to those under SD.

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