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EFFECT OF SOURCES AND DOSES OF NITROGEN ON CHLOROPHYLL CONTENTS OF GLADIOLUS CV. DHANVANTARI

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ABSTRACT

CAN at 40 g N/m2 gave highest chlorophyll contents (a, b and total) at 3- and 6- leaf stages of gladiolus cv. Dhanvantari. Increase in N doses increased total chlorophyll contents at 6-leaf stage. The trend of chlorophyll contents was similar at 6-leaf and spike emergence stages, except the concentrations. Urea provided minimum chlorophyll contents.

Key words : Nitrogen sources, gladiolus, chlorophyll

Nitrogen is an essential constituent of metabolically active compounds such as amino acids, proteins, enzymes, nucleic acids, RNA, nucleotides, purines, pyrimidines, coenzymes and hexose amines. It is a part of the chlorophyll molecule and of the photosynthetic enzyme rubisco which actively participates in protein synthesis (Salisbury and Ross, 1995). Its deficiency results in chlorosis of plants.

A number of factors like the form of nitrogen applied, the concentration and ratio of ammonium and nitrate, the presence of a particular cation or anion and physiological age of the plant influence the absorption and assimilation of nitrogen in horticultural crops (Barker and Mills, 1980). A close relationship is known to exist between applied nitrogen concentration, protein and chlorophyll content of leaves in plants. Rise in leaf chlorophyll concentration with increasing N levels was reported in a number of plants (Maynard, 1970; Subbiah and Ramanathan, 1982; Ogunlela *et al.*, 1984 and Damke, 1992). Hence the present investigation on gladiolus was carried out with different N sources if this has any response on chlorophyll contents on gladious.

MATERIALS AND METHODS

Corms of gladiolus cv. Dhanvantari were planted in a plot of 1.5 x 1.5 m^2 area during 1997-99. The 16 treatments consisted on 3 sources of nitrogen, viz., urea, ammonium sulphate and calcium ammonium nitrate (CAN), and 5 doses of nitrogen viz., 5, 10, 20, 30 and 40 g N/m^2 and a control. Nitrogen was applied in two split doses, i.e. the first half as basal dose along with 20 g P_2O_5/m^2 as single superphosphate, 20 g K_2O/m^2 as muriate of potash and 2 kg FYM/m^2, and the second half at 45 days after planting. Chlorophyll was extracted from the third leaf from the top of the plant, at three different stages of crop growth, viz., 3-leaf stage

(40 days after planting = DAP), 6-leaf stage (70 DAP) and at spike emergence (120 DAP) by non-maceration of tissue using dimethyl sulphoxide (DMSO) by the procedure of Hiscox and Israelstam (1979). Chlorophyll *a*, *b* and total chlorophyll were estimated according to Arnon (1949) and expressed as mg/g fresh weight. There were three replications per treatment and the pooled data of two years were analysed by Factorial Randoised Block Design (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

It's evident from Table 1 that application of CAN 40g N/m^2 resulted in the highest chlorophyll a (1.30 mg/g) and *b* contents (0.49 mg/g) in the third leaf of gladiolus at 3-leaf stage. This treatment was not significantly different from any of the treatments, except 5 and 10g N/m^2 through urea which were at par with the control resulting in the lowest chlorophyll a content of 0.72 mg/g. However, sources did not have any significant difference in chlorophyll *b* content of leaves. CAN at 40 g N/m^2 recorded 1.87 mg/g of total clorophyll content at 3-leaf stage but the difference was not significant with 30 g N/m^2 of CAN. CAN and ammonium sulphate resulted in higher total chlorophyll content than urea.

Comparison among the N sources revealed that ammonium sulphate application resulting in 1.17 mg/g chlorophyll a was significantly superior over urea (1.03 mg/g) and CAN (1.11 mg/g). Higher N doses, viz., 30 and 40 g N/m^2 resulted significantly in higher (1.19 and 1.21 mg/g, respectively) chlorophyll *a* content over the lower doses.

There was a progressive increase in total chlorophyll content at 6-leaf stage with increasing N doses. The highest dose resulted in the maximum (1.73 mg/g) total chlorophyll content, although it was as equally effective as 20-30 g N/m^2. The trend at 6-leaf stage and spike emergence stage with reference to chlorophyll *a*, *b* and total chlorophyll were similar to that of 3-leaf stage, except for the fact that there was an increase in the concentrations, followed by a decline in the same at spike emergence.

Considerably improved chlorophyll a,b and total chlorophyll content in the leaves with CAN application at all the three stages could be due to the increased calcium content in the leaf tissue. Calcium application defers the senescence of leaf discs through improved maintenance of chlorophyll and protein levels. Sharma (1996) was of the opinion that the presence of calcium slowed down the rate of chlorophyll degradation. Much of the calcium within the cytosol becomes reversibly bound to a small, soluble protein called calmodulin (Cheung, 1982) and activates the enzyme. Calcium is also essential for normal membrane function in all cells, probably as a binder of phospholipids to each other or to membrane proteins (Salisbury and Ross, 1995). The increasing supply of calcium generally accelerates nitrate absorption (Barker and Mills, 1980). The effect of cations like calcium on nitrate uptake may be to counter the negative charges on the root cell walls so that nitrate ions may migrate more closely to the plasmalemma and its uptake sites than they could in the absence of these ions (Elzam and Epstein, 1965). Nitrate in the presence of ammonium (as in case of calcium ammonium nitrate) has been reported to enhance plant growth and increase the total acquisition of nitrogen by plants (Mills *et al.*, 1976). Since nitrogen is an essential constituent of the chlorophyll molecule and of the enzyme rubisco present in the leaves which actively participates in photosynthesis (Salisbury and Ross, 1995) therefore this could also be a reason behind increased *a*, *b* and total

Table 1. Effect of N sources and doses on chlorophyll contents (mg/g) in the leaves of gladiolus cv. Dhanvantari

Treatment	Chlorphyll contents (mg/g) at :								
	3-leaf stage			6-leaf stage			spike emergence		
	a	b	Total	a	b	Total	a	b	Total
Urea (g N/m²)									
5	0.85	0.28	1.14	0.98	0.36	1.48	0.86	0.22	1.24
10	0.94	0.31	1.30	1.09	0.41	1.66	0.92	0.42	1.41
20	1.06	0.47	1.52	1.22	0.41	1.87	1.09	0.46	1.43
30	1.15	0.43	1.61	1.31	0.49	1.78	1.27	0.37	1.66
40	1.14	0.44	1.62	1.46	0.52	2.00	1.24	0.58	2.02
Ammonium sulphate = AS (g/m²)									
5	1.11	0.37	1.53	1.33	0.45	1.87	1.15	0.31	1.55
10	1.14	0.39	1.61	1.41	0.43	1.90	1.21	0.39	1.66
20	1.17	0.44	1.66	1.50	0.53	1.98	1.37	0.39	1.67
30	1.23	0.44	1.75	1.63	0.56	2.24	1.46	0.28	1.78
40	1.20	0.44	1.69	1.64	0.46	1.99	1.23	0.37	1.75
Calcium ammonium nitrate = CAN (g N/m²)									
5	1.10	0.34	1.28	1.26	0.47	1.78	1.12	0.43	1.63
10	0.98	0.33	1.48	1.41	0.52	1.98	1.29	0.43	1.78
20	1.10	0.37	1.52	1.53	0.51	2.15	1.34	0.41	1.79
30	1.17	0.37	1.63	1.60	0.53	2.02	1.35	0.57	1.99
40	1.30	0.49	1.87	1.68	0.62	2.19	1.38	0.54	1.98
Control	0.72	0.26	1.04	0.93	0.37	1.24	0.87	0.23	1.77
C.D. at 5%									
Source x Dose	0.26	N.S.	0.24	0.24	N.S.	0.35	0.32	0.14	0.37
C vs. T	0.25	0.16	0.37	0.25	0.11	0.37	0.33	0.17	0.41
N sources									
Urea	1.03	0.39	1.44	1.21	0.44	1.76	1.07	0.41	1.55
AS	1.17	0.42	1.65	1.50	0.49	2.00	1.29	0.35	1.68
CAN	1.11	0.38	1.55	1.49	0.53	2.03	1.29	0.48	1.83
CD at 5%	0.11	N.S.	0.16	0.11	0.05	0.16	0.14	0.08	0.18
N doses									
5	0.99	0.33	1.32	1.19	0.43	1.71	1.05	0.32	1.47
10	1.02	0.34	1.45	1.30	0.45	1.85	1.14	0.42	1.62
20	1.11	0.43	1.57	1.42	0.48	2.00	1.27	0.42	1.63
30	1.19	0.42	1.66	1.51	0.53	2.02	1.36	0.41	1.81
40	1.21	0.46	1.73	1.59	0.53	2.06	1.29	0.49	1.92
C.D. at 5%	0.14	0.09	0.22	0.14	0.06	0.20	0.10	0.10	0.23

C vs. T = Control vs. Treatments

chlorophyll contents in CAN-treated plants.

Among the three N sources, ammonium sulphate was next to CAN, while urea as N source had the least effect on chlorophyll content of leaves. The efficiency of ammonium

sulphate in increasing chlorophyll *a, b* and total chlorophyll contents could be because the NH_4^+ ion is adsorbed on the soil as exchangeable cations and is not as readily subject to leaching or gaseous loss (Parr, 1973) as urea.

Favourable effect of ammonium sulphate on enhanced chlorophyll contents could be attributed to the pesence of sulphur in the fertilizer. Sulphur is a constituent of amino acids (cysteine and methionine), vitamin B and coenzyme A, and participates in protein synthesis (Salisbury and Ross, 1995). It can be reasoned that due to enhanced protein synthesis with ammonium sulphate application, there was increase in the number and size of cells and chloroplasts, which resulted in enhanced chlorophyll contents. Although the mechanism involved in increasing sulphur uptake is poorly understood, sulphur uptake has been postulated as being associated with enhanced cation (HN_4^+) uptake (Blair *et al.*, 1970).

There was a progressive increase in chlorophylls *a, b* and total chlorophyll with increasing doses of applied N. The probable reason for this could be increased protein synthesis as a result of abundance of available N, resulting in greater number and size of chloroplast in the leaves. The present findings are in conformity with the results of Maynard (1970) in spinach, Subbiah and Ramnathan (1982) in amaranthus, Ogunlelea *et al.* (1989) in *Brassica napus*, and Damke (1992) in rose.

An increase in chlorophyll a, b and total chlorophyll from the lowest level at 3-leaf stage to the maximum level at 6-leaf stage was noted, followed by a decline in the same at spike emergence. The lowest chlorophyll contents were found at 3-leaf stage because at this stage, the N taken from the soil was utilized almost exclusively for vegetative growth. There was maximum chlorophyll content in the leaves at 6-leaf stage because by this time the plant and leaves had almost completed its above ground vegetative growth, therefore nitrogen could be diverted for the production of chloroplasts. The proteins in leaves are mostly present as the abundant photosynthetic enzyme rubisco. At spike emergence, this enzyme was hydrolysed by proteinases, therefore photosynthetic activity decreased considerably during flowering in gladiolus. This had been called a "self destruct" phenomenon because transport of N from the vegetative organs occurs partly at the expense of rubisco. In plants growing in soils in which nitrogen is not abundant, the hydrolysis of protein and transport of nitrogen from the leaves to the developing organs is essential. Chloropylls also disappear from leaves as proteins are degraded and the nitrogen in these molecules is apparently also transported to the sinks, i.e. reproductive organs and corms.

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