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PROTEASE ACTIVITY IN RELATION TO MOBILIZATION OF LEAF NITROGEN DURING POD DEVELOPMENT IN IRRIGATED AND UNIRRIGATED CHICKPEA CULTIVARS

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SUMMARY

Protease activity in relation to leaf nitrogen mobilization was examined in four chickpea (*Cicer arietinum* L.) cultivars (Pusa 256, Pusa 72, Pusa 267 and Pusa 372) grown in unirrigated and irrigated field conditions. The irrigation treatment consisted of two additional irrigations applied at flowering and pod filling stages. A decrease in the content of leaf nitrogen was observed during pod development. Leaf nitrogen was higher in irrigated than in unirrigated plants. The activity of protease at pH 4 was higher than at pH 7 and was less in irrigated than in unirrigated plants. These findings are discussed in relation to decrease in harvest index by irrigation commonly observed in this crop under north Indian conditions.

***Key words*: Chickpea, harvest index, irrigation, leaf nitrogen, protease.**

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop in India. The yield of this crop has remained low in spite of concerted efforts by breeders (Jain 1988). This poor yield has been partly attributed to the fact that chickpea is mostly grown on relatively dry land of low fertility with extremely low levels of agronomic management. It was, therefore, suggested that in order to increase the productivity of this crop, management support in terms of irrigation needs to be provided (Jain 1988). However, only pre-sowing irrigation was found to be beneficial at Delhi (Choudhury *et al.* 1972). For maximum yield, it is necessary to irrigate once after planting, at the pre-flowering stage, if no winter rains are received until flowering (Yadav 1975, Singh *et al.* 1984, Kaushik and Chaubey 1999). Irrigation applied after flowering may even decrease the seed yield (Parihar 1990, Nanda and Saini 1992). Irrigation after flowering, therefore, does not generally maximize chickpea yield under north Indian condition as it increases vegetative growth and decreases harvest index and nitrogen harvest index (Khanna-Chopra and Sinha 1987, Singh 1991, Nanda and Saini 1992, Sinha 1995). Understanding the physiological basis of such a response would possibly help in developing irrigation responsive chickpea varieties.

Chickpea being a protein rich grain legume accumulates large quantities of N in seeds. Since, the N requirement is not met by current sources of N, vegetative N, particularly leaf N, is mobilized for seed development in grain legumes (Sinclair and DeWit 1975, Evans 1982, Ghildiyal and Sirohi 1986, Ghildiyal *et al.* 1987, Mitra and Ghildiyal 1988, Singh 1990). Hooda *et al.* (1990) observed that in chickpea about 55-58% of the seed N requirement was met by mobilization from vegetative parts; leaves contributed 39% of seed N. The mobilization of vegetative N is, therefore, of considerable significance for seed yield in chickpea (Sinha 1995). It was reported earlier that leaf nitrogen including Rubisco is mobilized during pod development in chickpea and irrigation decreased this mobilization (Pandey *et al.* 2001).

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Proteases catalyze the hydrolysis of proteins leading to mobilization and translocation of N from vegetative tissues. The present study, therefore, attempted to analyze the protease activity in relation to mobilization of leaf N in irrigated and unirrigated chickpea cultivars.

MATERIALS AND METHODS

Chickpea (*Cicer arietinum* L.) cvs. Pusa 256, Pusa 72, Pusa 267 and Pusa 372 were grown in field under unirrigated and irrigated field conditions. Fertilizers were applied to the soil at the rate of 15, 60 and 60 kg ha^{-1} of N, P and K, respectively. Seeds were inoculated with *Rhizobium* culture prior to sowing. Plant to plant spacing was maintained at 30 cm between rows and 12 cm within rows. A pre-sowing irrigation was applied to the entire field. The irrigation treatment consisted of two additional irrigations applied at the flowering and pod filling stages. The first flower appeared around 90 days after sowing (DAS) and more than 50% of flowering had taken place by 105 DAS. Irrigation at the flowering and post flowering stages were applied at 105 and 125 DAS. Samples for leaf N and dry weight of different plant parts from irrigated and unirrigated plants were taken at 75 DAS (pre-flowering), 112 DAS (flowering) and 132 DAS (postflowering). Yield components were determined at harvest. The total N content was determined in the dried leaf samples using nitrogen analyzer (Gerhardt Bonn). Protease activity was determined in the uppermost fully expanded leaves of unirrigated and irrigated plants 112 DAS (flowering) and 132 DAS (post-flowering). Fresh weight/dry weight ratio of comparable leaves was used to expressed activity on dry weight basis. Protease activity was determined at pH 4 and pH 7 following the method described by Nair *et al.* (1978). There were a minimum of three replications for each determination.

RESULTS AND DISCUSSION

The leaf nitrogen content decreased after flowering during pod development in all the cultivars under both unirrigated and irrigated conditions (Table 1). Irrigated plants, however, had significantly higher leaf N content than unirrigated plants (Table 1). Protease activity in the leaves of all the chickpea cultivars was higher at pH 4 than at pH 7. At pH 4 protease activity was less in irrigated than in unirrigated plants in all the cultivars (Table 2). The harvest index (HI) was decreased by irrigation in all the cultivars of chickpea (Table 3). The extent of decrease in HI by irrigation varied among cultivars. Pusa 72 showed greater reduction in HI by irrigation compared to other cultivars. This cultivar also showed a greater reduction in acid protease activity by irrigation at flowering stage.

The present study indicates that in chickpea cultivars leaf N is mobilized during seed development and irrigation decreases this mobilizaton. The decrease in N mobilization by irrigation was found to be associated with a decrease in the protease activity at pH 4. No such relationship was observed with protease activity measured at pH 7.

The mobilization of vegetatively stored N for seed development has been reported for serveral grain legumes including chickpea (Evans 1982, Ghildiyal and Sirohi 1986, Ghildiyal *et al.* 1987, Khanna-Chopra and Sinha 1987, Mitra and Ghildiyal 1988). It has been suggested that, because of the higher protein content of the seeds in grain legumes their N requirement for seed development is very high and demands N translocation from vegetative tissues (Sinclair and DeWit 1975, Singh 1990). In the present study, a decrease in the mobilization of vegetative N by irrigation appeared to be due to a decrease in protease activity measured at pH 4. Cultivar showing a greater decrease in protease activity by irrigation also showed a greater decrease in HI.

Vacuoles are rich in hydrolytic enzymes. The proteases they contain play a role in protein degradation (Spremulli 2000). In unirrigated plants, because of the possible oxidative stress, the permeability of vacuolar membrane may be affected. The vacuolar proteases may, therefore, possibly leak into the cytoplsm and hydrolyse both soluble proteins and proteins of chloroplast and mitochondrial membranes (Salisbury and Ross 1992). Chloroplasts contain approximately 50% of the protein found in photosynthetic tissues, so protein degradation must occur in chloroplasts. It has been shown that Rubisco is mobilized during pod development in chickpea (Pandey *et al.* 2001). One of the proteases observed in chloroplasts is serine protease. This protease is believed to play an important role in degrading chloroplast proteins during leaf sensescence (Spremulli 2000). Specific protease involved in degradation of leaf protein is not clear from the present study. However, it appeared to be an acid protease, as

Table 1. Nitrogen content in the leaves (mg plant^{-1}) of chickpea cultivars grown under unirrigated and irrigated field contitions

Stage	Treatment	Cultivars			
		P 256	P 72	P 267	P 372
Pre-flowering	Unirrigated	16.58±0.11	18.11±0.61	10.86±1.23	15.32±0.82
Flowering	Unirrigated	72.24±1.35	74.09±2.14	51.09±0.13	66.31±0.19
	Irrigated	74.19±0.37	90.49±1.22	67.28±3.61	70.80±1.16
Post-flowering	Unirrigated	6.70±0.03	8.28±0.26	10.57±0.05	9.72±0.06
	Irrigated	15.94±0.54	14.09±0.26	14.34±0.15	14.66±0.07

Table 2. Protease activity in the leaves of chickpea cultivars grown under unirrigated and irrigated field conditions.

Cultivar	Treatment	Flowering		Post-flowering	
		pH4	pH7	pH4	pH7
		(μmol glycine equivalent g^{-1} fw h^{-1})			
P 256	Unirrigated	5.39±0.44	2.19±0.01	3.22±0.68	0.89±0.13
	Irrigated	4.49±0.01	0.75±0.01	1.85±0.26	0.86±0.10
P 72	Unirrigated	6.30±0.54	0.98±0.22	1.93±0.02	1.21±0.24
	Irrigated	2.63±0.90	1.13±0.25	1.73±0.08	1.43±0.04
P 267	Unirrigated	3.59±0.59	1.05±0.26	3.35±0.45	0.56±0.06
	Irrigated	2.25±0.14	2.25±0.87	1.61±0.24	0.74±0.11
P 372	Unirrigated	6.98±0.88	1.46±0.06	1.57±0.37	0.60±0.20
	Irrigated	3.74±0.66	2.75±1.02	1.57±0.14	0.96±0.05
		(μmol glycine equivalent g^{-1} dry w h^{-1})			
P 256	Unirrigated	24.39±1.99	9.90±0.04	13.41±2.83	3.72±0.54
	Irrigated	19.69±0.04	3.28±0.04	9.25±1.30	4.30±0.50
P 72	Unirrigated	25.60±2.19	3.98±0.89	9.19±0.09	5.79±1.14
	Irrigated	12.64±4.32	5.43±1.20	8.48±0.39	7.00±0.19
P 267	Unirrigated	14.77±2.42	4.32±1.06	12.22±1.64	2.04±0.21
	Irrigated	11.08±0.68	11.08±4.28	8.21±1.22	3.81±0.56
P 372	Unirrigated	26.64±3.35	5.57±0.22	7.00±1.64	2.46±0.82
	Irrigated	17.47±3.08	12.85±4.76	7.00±0.62	4.28±0.22

Table 3. Seed yield, total dry matter and harvest index of chickpea cultivars grown under unirrigated and irrigated field conditions

Cultivar	Treatment	Seed yield (g plant^{-1})	Total dry matter (g plant^{-1})	Harvest index (%)	Per cent decrease in HI by irrigation
P 256	Unirrigated	6.17±1.08	14.76±0.48	41.80±1.21	37.10
	Irrigated	5.72±0.22	21.75±0.63	26.29±0.41	
P 72	Unirrigated	6.36±0.15	16.33±0.34	38.94±0.82	52.31
	Irrigated	4.13±0.12	22.24±0.26	18.57±0.42	
P 267	Unirrigated	5.30±0.45	16.64±0.79	31.85±1.12	36.04
	Irrigated	3.71±0.16	18.21±0.26	20.37±0.62	
P 372	Unirrigated	5.66±0.26	16.02±0.75	35.33±0.84	39.48
	Irrigated	3.06±0.17	14.31±0.58	21.38±0.97	

protease activity measured at pH 4 showed a good correlation with mobilization of leaf N in relation to irrigation. The activity of this protease decreased in irrigated plants, which showed lesser mobilization of leaf N. It has been observed that irrigation suppresses flowering and pod number thus decreasing the N requirement of the sink (Pandey *et al.* 2001). The N was, therefore, retained at a higher level in the vegetative tissues. The decrease in protease activity by irrigation could, therefore, be possibly regulated through hormonal signal from the sink.

The mobilization of leaf nitrogen for seed development decreases leaf photosynthesis, induced senesence and restricts the duration of seed fill period has been observed in mungbean and pigeonpea (Luthra *et al.* 1983, Rao and Ghildiyal 1985, Ghildiyal and Sirohi 1986, Mitra and Ghildiyal 1988). In chickpea, on the other hand, decrease in mobilization of N by irrigation led to a decrease in HI. Consequently, chickpea plants were not able to utilize the beneficial effect of irrigation for seed yield. Question arises, whether we need such chickpea genotypes in which protease remained active in irrigated plants. Considering that low yields of chickpea were ascribed to poor realization of potential sinks because of lower mobilization of fixed N (Sinha 1977, 1995), such a requirement may not be ruled out.

REFERENCES

Choudhury, S.L., Sharma, B.M., Bhatia, P.C. and Ram, S. (1972). When and where do *rabi* pulses pay more? *Indian Farming* **21**: 28-29.

Evans, J. (1982). Symbiosis, nitogen and dry matter distribution in chickpea (*Cicer arietinum* L.). *Exp. Agric.* **18**: 339-351.

Ghildiyal, M.C. and Sirohi, G.S. (1986). Nitrogen utilization during growth and development in mungbean. *Indian J. Exp. Biol.* **24**: 124-126.

Ghildiyal, M.C., Uprety, D.C. and Sirohi, G.S. (1987). Influence of pods on senescence of leaves in bengal gram. *Sci. Cult.* **53**:318-320.

Hooda, R.S., Sheoran, I.S., Singh, Randhir and Singh, R. (1990). Partitioning and utilization of carbon and nitrogen for dry matter and protein production in chickpea (*Cicer arietinum* L.) under control and stress conditions. *Indian J. Exp. Biol.* **28**: 280-283.

Jain, H.K. (1988). Production of pulses in India–past trends and future potential. In: B. Baldev, S. Ramanujam and H.K. Jain (eds.), Pulse Crops, pp. 52-91, IBH Publications, New Delhi.

Kaushik, M.K. and Chaubey, A.K. (1999). Studies on irrigation rquirement of chickpea (*Cicer arietinum*). *Indian J. Agron.* **44**: 367-369.

Khanna-Chopra, R. and Sinha, S.K. (1987). Chickpea : physiological aspects of growth and yield. In: M.C. Saxena and K.B. Singh (eds.), The Chickpea, pp. 163-190, CAB International, Wallingford, UK.

Luthra, Y.P., Sheoran, I.S. and Singh, R. (1983). Ontogenetic interactions between photosynthesis and nitrogen fixation in pigeonpea. *Ann. Appl. Biol.* **103**: 549-556.

Mitra, S. and Ghildiyal, M.C. (1988). Photosynthesis and assimilate partitioning in mungbean in response to source sink alteration. *J. Agron. Crop Sci.* **160**: 303-308.

Nair, T.V.R., Grover, H.L. and Abrol, Y.P. (1978). Nitrogen metabolism of the upper three leaf blades of wheat at different soil nitrogen levels. *Physiol. Plant* **42**: 293-300.

Nanda, R. and Saini, A.D. (1992). Effect of restricted soil moisture on yield and its attributes in chickpea. *Indian J. Plant Physiol.* **35**: 16-24.

Pandey, R., Sharma-Natu, P. and Ghildiyal, M.C. (2001). Irrigation decreases loss of leaf nitrogen and Rubisco during pod development in chickpea. *J. Agron. Crop Sci.* **187**: 177-183.

Parihar, S.S. (1990). Yield and water use of chickpea (*Cicer arietinum*) as influenced by irrigation and phosphorus. *Indian J. Agron.* **35** : 251-257.

Rao, T.R.K. and Ghildiyal, M.C. (1985). Analysis of photosynthetic source and sink relationship in mungbean (*Vigna radiata* (L.) Wilczek). *Indian J. Plant Physiol.* **28**: 135-144.

Salisbury, F.B. and Ross, C.W. (1992). Plant Physiology, IV Edition. Wadsworth Pub. Co., Belmont, California.

Sinclair, T.R. and DeWit, C.T. (1975). Photosynthate and nitrogen requirements for seed production by various crops. *Science* **189**: 565-567.

Singh, G., Kalra, N., Sastry, P.S.N., Baldev, B., Murti, G.S.R.K. and Singh, G. (1984). Water use in chickpea as affected by irrigation scheduling in semiarid. *Trans. Indian Soc. Desert Techn.* **9**: 58-61.

Singh, P. (1991). Influence of water deficits on phenology, growth and dry matter allocation in chickpea (*Cicer arietinum* L.). *Field Crops Res.* **28**: 1-5.

Singh, R. (1990). Assimilation and partitioning of carbon and nitrogen during growth and development of grain legumes. In: S.K. Sinha, P.V. Sane, S.C. Bhargava and P.K. Agrawal (eds.), Proc. Int. Cong. Plant Physiol. 1, pp. 261-267. Soc. Plant Physiol. & Biochem., New Delhi.

Sinha, S.K. (1977). Food Legumes : Distribution, Adaptability and Biology of Yield. FAO Plant Production and Protection Publication 3, FAO, Rome.

Sinha, S.K. (1995). Status of pulses in the Asia Pacific region-production, research and development. In: S.K. Sinha and R.S. Paroda (eds.), Production of Pulse Crops in Asia and Pacific Region. FAO Regional Office for Asia and Pacific Publication 8, pp. 35-42, FAO, Bangkok.

Spremulli, L. (2000). Protein synthesis, assembly and degradation. In: B.B. Buchanan, W. Gruissem and R.L. Jones (eds.), Biochemistry and Molecular Biology of Plants, pp. 412-450. American Society Plant Physiologists, Rockville, Maryland.

Yadav, J.S.P. (1975). Annual Report of ICAR All India Coordinated Scheme for Research on Water Management and Salinity, CSSRI, Karnal, India.