SALT TOLERANCE PROTEINS IN DEVELOPING CHILLI FRUIT

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Abstract: The influence of Nacl on different quality attributes such as protein and protease of the Chilli Fruits were investigated during the developmental stages. Electrophoresis analysis of total soluble protein (SDS-PAGE) profile was carried out in order to evaluate the response of chilli fruits to salt stress. Protein content increased with attainment of fruit maturity SDS-PAGE analysis has revealed that plant grown under Nacl (50 and 100mM) showed induction or repression in the synthesis of few polypeptides in green and red fruits. This increase in protein content with increase in fruit maturity indicates that these concentrations of Nacl enhance protein synthesis which increases the ability to cope with salinity.

Keywords: Chilli Fruit, Nacl, Protein Analysis

INTRODUCTION

In India, chilli is an important commercial crop grown as condiment cum vegetable crop in an area of 9.17 lack hac. With a production of 7.79 lakh tones (Anon., 1). There is about 8 million ha of salt affected soil (saline and sodic) in India (Mangal et al., 1990). Critically the problem of salinization is increasing, often due to bad agriculture practices. Increased synthesis of a wide variety of proteins occurs in response to salt stress. Proteolytic enzymes also called proteases, are the enzymes that catalyse the hydrolytic cleavage of specific peptide bonds in their target proteins. However, plants subjected to salt stress showed increased levels of total free amino acids (Dubey & Pessarakli, 1995). The present work was carried out to investigate the effect of salinity (50 and 100mM NaCI) on protease, protein and protein profile of fruits of Capsicum annum cv. Pusa Jwala.

MATERIAL AND METHOD

Capsicum seeds were obtained from Indian Agricultural Research Institute, New Delhi. Prior to experimentation they were selected for uniformity of size and mass.

Sowing and salt stress treatments

The seeds were sown in 6 sets of poly bags after filling them with pre analysed garden soil. Soil was air-dried, mixed thoroughly. The soil has pH 7.17 ±0.1, organic carbon 0.29 ±0.00% C ± SD, water holding capacity 65.5, phenolics 6.38 ±1.69 eq g fw^{-1} ±SD, Na+ 0.33 ± 0.00 K+0.09 ±0.00, Ca^{++}1.03 ±0.06 meq/ g d wt.

The poly bags with untreated distilled water irrigated soil were used as control and with 50 and 100 mM NaC1 treatment were used for developing salt stress. 10 seeds of chilli were sown in each poly bag. Subsequent irrigation was given using distilled water. After germination, one seedling was retained in each poly bag. Finally fruits were harvested at different levels of maturity (green 26 days after anthesis and red 40 days after anthesis) for experiment.

Protein

The total soluble protein concentration was determined in fresh tissues according to Lowry et al. (1951).

Protease

Protease was estimated according to Green and Newuath, 1954.

Protein profiling

The extracted protein samples were fractionated by sodium dodecy1 sulphate -polyacrylamide gel electrophoresis (SDS-PAGE). A solution of 12% SDS-polyacrylamide slab gel was prepared according to the method of Laemmli (1970) and equal volumes of proteins extracted were loaded. Scanning of the separated protein bands was analysed by the Gel Documentation System.

RESULTS AND DISCUSSION

An increase in Nacl concentration has been recorded to exhibit a stimulatory effect on protein content in different parts of fruits. Increase in salt concentration from 50 to 100 mM Nacl caused decrease in protease activity in fruit wall and seeds of green fruit stage but reverse in placenta compared with those of untreated fruits. With attainment of maturity, protease activity increased with salt stress compared to control (Fig. 2).

Generally, if protease activity is high then protein content is low, but at 50 mM Nacl treated sets synthesis of new proteins is indicated even with higher protease activity in seeds of green and red fruits and also in placenta of red fruit (Table 1, 2).

Proteases are enzymes that catalyse the hydrolytic cleavage of specific peptide bonds in their target proteins. Protease inhibitors regulate the activity of

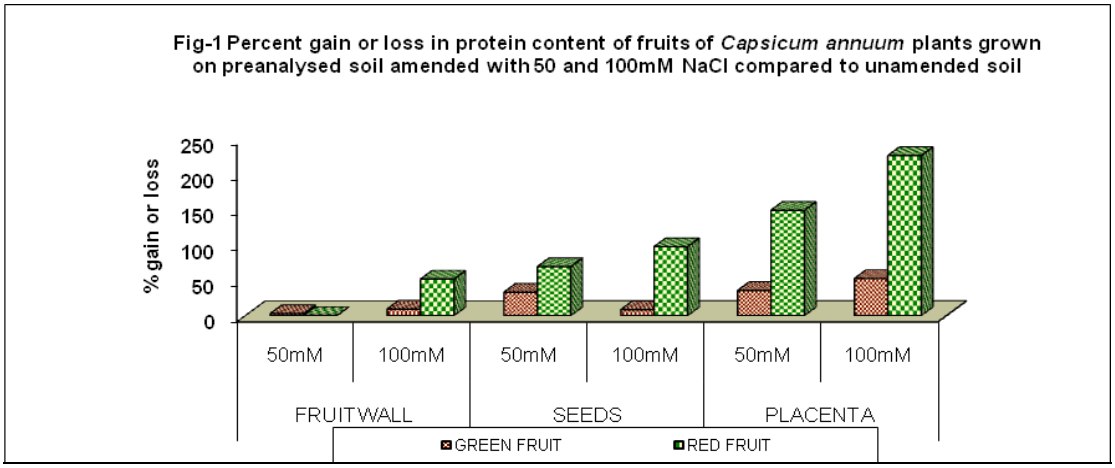
protease. Plant protease inhibitors generally small protein that have mainly been described as occurring in storage tissues, such as tubers and seeds. They are also induced in plants in response to injury. Based on the present study it may be possible that in new protein some may be protease inhibitor that check the protease activity in presence of higher protease the amount of protein was higher. Protease inhibitors are key players in the endogenous defense system, as they help regulate and balance protease activities. (Habib et al., 2007).

This increase in protein content with increase in fruit maturity indicates that these concentration of NaC1 enhance protein synthesis which increases the ability to cope with salinity.

It has been observed in the present work that three protein bands (M.wts: 26. 34.19 and 34.5 kDa) appeared in fruits of salts stressed plants. A protein band of molecular weight 34.19 kDa was denovo synthesized in placenta of green and red fruit in response to 100 mM Nacl (Table Fig 3), while protein band of molecular weights 26 and 34.5 kDa were newly synthesized as common salt adaptive protein in 100 mM Nacl treated fruits exhibits sustenance of plants to salt stress through either completing or synthesis of new proteins. These proteins may belong to chaperonin category protecting the required proteins from degradation.

34 kDa polypeptide has also been identified and reported in potato plants subjected to water deficit (Pruvot et al 1996 a). Since 26 kDa protein is specifically synthesized and accumulated in cells undergoing osmotic adjustment to salt stress, this protein is named osmotin (Singh et al., 1987). Osmotins have properties similar to chaperons. Under 50mM Nacl treatment more degradation of high molecular weight proteins is observed than under 100mM Nacl treatment. Besides, red fruits and their placenta appear to be more tolerant than green fruits. The most susceptible part appears to be the seeds (storage organ) with highest number of degraded proteins under 50 mM Nacl treatment.

This investigation points at accumulation of certain proteins to be instrumented in amelioration of the adverse effects of salinity stress. Also that 50mM Nacl induces shock responses against which all the tolerance indicators do not necessarily get synthesized. However, 100mM Nacl stabilizes and restores the degrading proteins with the help of synthesis of new high and low molecular weight proteins. Thus 50mM Nacl induces stress but 100 mM Nacl exhibits restoration tolerance from stress. 150 mM Nacl did not lead to germination, indicating it to be lethal dose for Capsicum not allowing the tolerance proteins to play their role.



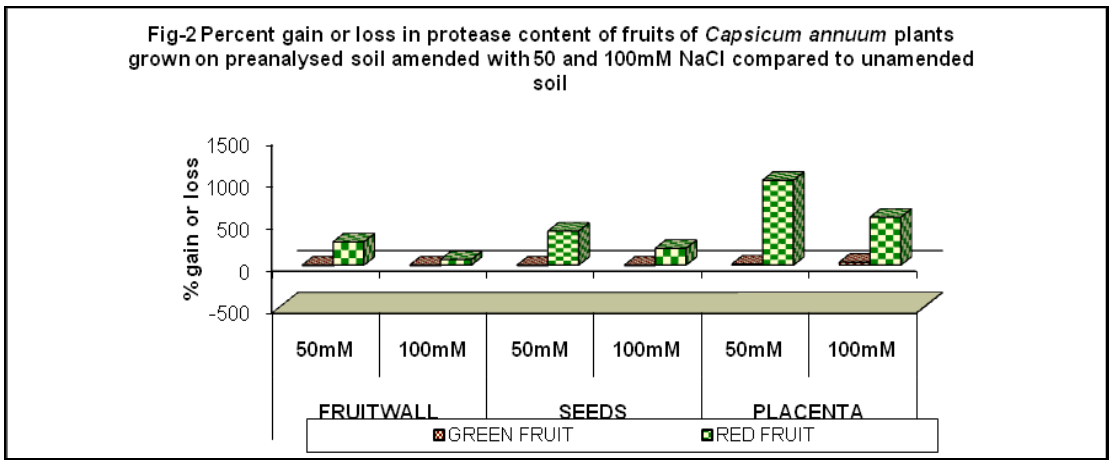


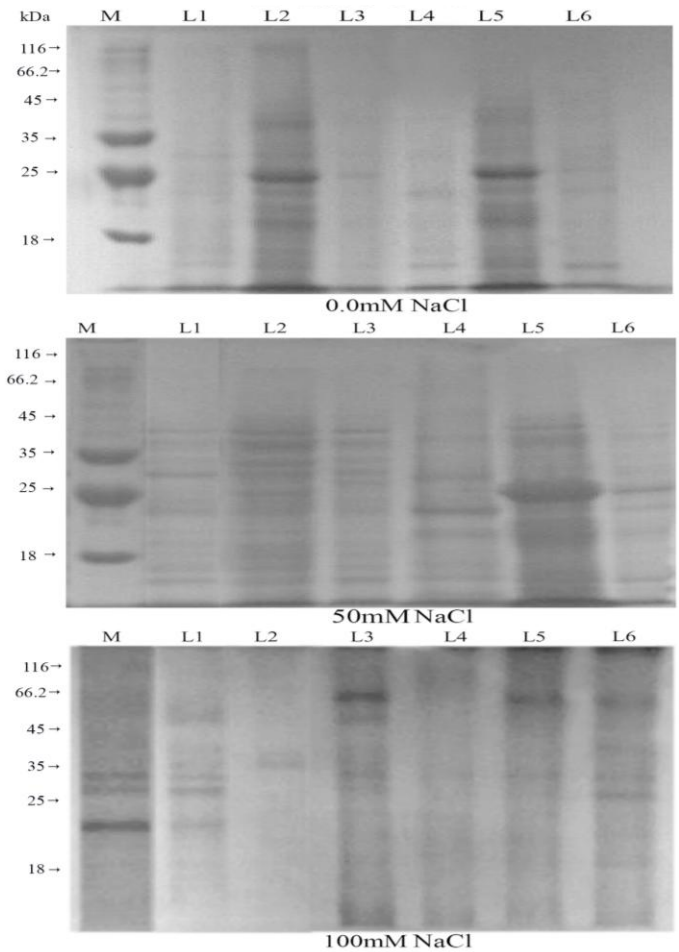
Table-1. Protein (mg casein eq gfw^{-1} ±SD) in fruits of C. annuum cv. P.J. under different salt concentration (mM Nacl)

S.N.	FRUIT STAGE	FRUIT WALL			SEED			PLACENTA		
		DW	50mM	100mM	DW	50mM	100mM	DW	50mM	100mM
1.	GREEN FRUIT	58.77 ±1.16	60.53 ±0.53	64.34 ±0.53	62.7. ±0.53	83.60 ±0.76	68.04 ±1.41	117.04 ±2.14	158.85 ±5.37	178.61 ±3.80
2.	RED FRUIT	91.2.5 ±1.31	91.45 ±0.43	138.43 ±3.83	100.82 ±4.38	170.75 ±4.56	199.30 ±2.92	163.66 ±3.16	407.76 ±7.53	534.23 ±2.46

Table-2. Protease (mg tyrosine eq gfw^{-1} ±SD) in fruits of c. annuum cv. P.J. under different salt concentration (mM Nacl)

S.N	FRUIT STAGE	FRUIT WALL			SEED			PLACENTA		
		DW	50mM	100mM	DW	50mM	100mM	DW	50mM	100mM
1.	GREEN FRUIT	5.37 ±0.05	4.95 ±0.12	4.98 ±0.21	5.49 ±0.06	5.19 ±0.04	5.223 ±0.04	9.95 ±0.11	11.15 ±10.00	13.33 ±0.55
2.	RED FRUIT	6.55 ±0.16	24.58 ±0.28	10.80 ±0.28	6.55 ±0.04)	33.01 ±0.73	19.55 ±1.46	14.49 ±1.52	160.43 ±2.14	96.79 ±1.04

Plate - 1



SDS-PAGE ANALYSIS OF PROTEIN PATTERN OF FRUITS OF *Capsicum annuum* CV. P.J. PLANTS TREATED WITH 0.0mM, 5OmM AND 100mM NaCl.
LANE(M) -PROTEIN MARKER
L(1) -FRUIT WALL (G) L(4) -FRUIT WALL(R)
L(2) -SEEDS (G) L(5) -SEEDS (R)
L(3) -PLACENTA (G) L(6) -PLACENTA(R)
(G) -GREEN FRUITS,(R) - RED FRUITS

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