INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–4–653–659
http://www.ijab.org

Physiological Responses of Wheat to Salinity Alleviation by Nicotinamide and Tryptophan

HALA M.S. EL-BASSIOUNY
Botany Department, National Research Centre, Dokki, Giza, Egypt
Corresponding author's e-mail: lollah43@hotmail.com

ABSTRACT

Two field experiments were carried out in the saline soil, during two successive seasons, to study the effect of foliar application of nicotinamide or tryptophan on improving wheat salt tolerance under saline condition. Application of nicotinamide or tryptophan resulted in significant increase in plant growth and grain yield, concomitantly with an increase in the level of IAA, GA_3, cytokinins, photosynthetic pigments and a decrease in ABA content. Moreover, nicotinamide or tryptophan reduced Na^+ and Cl^- content and increased K^+, Ca^{2+}, Mg^{2+} and P in the wheat shoots. Quantitative and qualitative changes in protein patterns were obtained in response to nicotinamide or tryptophan application relative to the plants received salt only. The previous changes were discussed in relation to roles of nicotinamide or tryptophan in enhancing wheat salt tolerance.

Key Words: Nicotinamide; Tryptophan; Wheat; Endogenous phytohormone; Protein

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the important cereal crops of high nutritive value in the world as well as in Egypt. The grains of wheat contain large amounts of proteins, carbohydrates in addition to some minerals and vitamins.

Salinity is one of the major problems of agriculture in aird and semiarid regions. The accumulation of salts in soil may be attributed to the high ground water table accompanied with poor drainage. Soil salinity has, therefore a great impact on decreasing yield potentials of the cultivated crops. Crop yields start declining when EC of the soil solution goes above 4 dS m^{-1} (Sairam *et al*., 2002). Addition of salts to water lowers its osmotic potential, thereby decreasing its availability to root cells. Thus, salt stress exposes the plant to secondary osmotic stress, which implies that physiological responses, invoked by drought, can also be observed under salt stress (Sairam *et al*., 2002). Growth reduction of vascular plants at high salinity levels may be attributed to the reduction in photosynthetic rate of plant tissues, which could arise from adverse effects of Na^+, Cl^- and water stress on metabolism (Munns, 2002).

Endogenous hormonal levels of plants were reported to undergo various and rapid changes under water stress and salinity (Shakirova, *et al*., 2003; Azooz *et al*., 2004). One approach to minimize the adverse effect of salinity on plants is the use of growth regulating substances, which have been reported to increase salt tolerance of plants (Saker, 1996).

Nicotinamide is a stress-associated compound that can induce and regulate secondary metabolic accumulation and/ or the manifestation of defense metabolism in plants (Berglund & Ohlsson, 1995). Nicotinamide might be a link between various types of stress, which leads to an increased frequency of DNA strand breaks, and plant defensive gene transcription (Berglund, 1994).

Tryptophan is the major precursor of IAA in most organisms (Ramaih *et al*., 2003). Steif (1988) found that stress decreased the tryptophan synthesis alpha monomers, which was gradually dissociated from oligomers. This in turn produced less active isoenzymes, reduced biosynthesis of L. tryptophan and consequently that of IAA. Martens and Frankenberger (1994) and Sarwar and Frankenberger (1994) indicated that the tryptophan treatment increased growth rate and yield of wheat and *Zea mays*, respectively. Wyszkowska (1999) found that tryptophan increased K^+, N and Ca^{2+} in different plant organs.

The aim of the present work was to study the influence of foliar spraying of nicotinamide and tryptophan on counteracting the deleterious effects of salinity on wheat. For this the growth, yield, endogenous phytohormones, photosynthetic pigments, nutrients and protein profile of wheat plants cultivated in high salinity were determined.

MATERIALS AND METHODS

Two field experiments were carried out in two successive seasons (2002/2003 & 2003/2004) at Abo-Mady area, North Delta Dakahlei Governorate, Egypt. The mechanical and chemical analysis of the experimental field soil is presented in Table I.

A split plot design was followed with four replicates. The experimental area was divided into three plots: the main plots were assigned to three treatments; control and two treatments (Nicotinamide, vitamin B; amino acid, tryptophan). The sub-plot received 25, 50 and 100 mg L^{-1} of

each chemical.

Caryopses of wheat (*Triticum aestivum* L., cv. Giza 168) were obtained from the Agricultural Research Centre, Giza, Egypt and sown on 15 November in the both seasons. Plot area was 12 m^2 (4x3 m), in rows four meters long and 25 cm apart. Before sowing, calcium superphosphate (15.5% P_2O_5) at 100 kg fed^{-1}, ammonium nitrate (33.5% N) at 100 kg fed^{-1} at three equal doses, and potassium sulfate (48-52% K_2O) at 50kg fed^{-1} at two equal doses were added to the soil. The plants were grown under natural growth conditions: 12 h light period, 65%-70% relative humidity, day/night temperatures of 24-18/20-14°C. Three concentrations (25, 50, 100 mg L^{-1}) of nicotinamide or tryptophan were applied foliarly twice. The first and second sprays were done 30 and 45 d after sowing, respectively.

After 75 d of sowing, the growth parameters, photosynthetic pigments, protein profile, endogenous hormones (IAA, GA_3, ABA & Cytokinins) and minerals contents (K^+, Na^+, Ca^{2+}, Mg^{2+}, P & Cl^-) were determined. The components were determined after 160 d of sowing.

Chemical analysis. Photosynthetic pigments (chl. a, chl. b and carotenoids) were determined Spectrophotometerically (Metzner *et al.,* 1965). Total carbohydrate percentage was determined in the grains as described by Younis *et al.* (1969). Protein percentage was determined according to the method of Bradford (1976). K^+, Na^+, Mg^{2+}, Ca^{2+}, P and Cl^- were determined according to the method described by Chapmen and Pratt (1978).

Determination of IAA, GA_3 and ABA were carried out by Liquid chromatography (GLC). Their extraction was carried out based on Shindy and Smith (1975) and methylation according to Vogel (1975). Cytokinin was extracted using the method of the previous acidic hormones and were quantified by HPLC (Muller & Hilgenbery, 1986).

Electrophoretic profile of wheat shoots proteins was determined by SDS–PAGE (Laememli, 1970), with related polypeptide maps; molecular protein markers, percentage of band intensity; molecular weight and mobility rate of each polypeptide to standard markers using gel protein analyzer version 3 (Media Cyberne Tice, USA).

Data were statistically analyzed according to Snedecor and Cochran (1980). Combined analysis of the two seasons was made and treatment means were compared using LSD test at 5% level of probability.

RESULTS AND DISCUSSIONS

Growth and yield components. The results in Table II indicate that exogenous application of nicotinamide improved growth and yield, 50 mg L^{-1} was more effective. Our results are in agreement with those of Balki and Padole (1982) and Foda (1987) who found that treating wheat caryopses with nicotinamide mitigated the deleterious effect of salinity on plant growth and yield. Similarly, Hathout *et al.* (1993a) report an increased tomato growth and yield in response to nicotinamide treatment.

Foliar application of tryptophan to wheat plants significantly increased plant growth (Table II). Maximum increase was obtained by 25 mg L^{-1} tryptophan (except plant height). Grain yield was increased as a result of tryptophan treatments, greater increase was obtained with 50 mg L^{-1}. Similar findings were obtained in different plant species in response to tryptophan application (Frankenberger *et al.,* 1990; Marten & Frankenberger, 1994; Arshad *et al.,* 1995).

Data presented in Fig. 1 showed that the percentage of carbohydrates in dry produced grains increased by nicotinamide treatments, more so with 50 mg L^{-1} (21%). Protein percentage was not significantly increased. Foda (1987), Sharaf El-Din *et al.* (1987) and Mohamed *et al.* (1989) found that nicotinamide increased the level of carbohydrates of wheat plant in different plant organs.

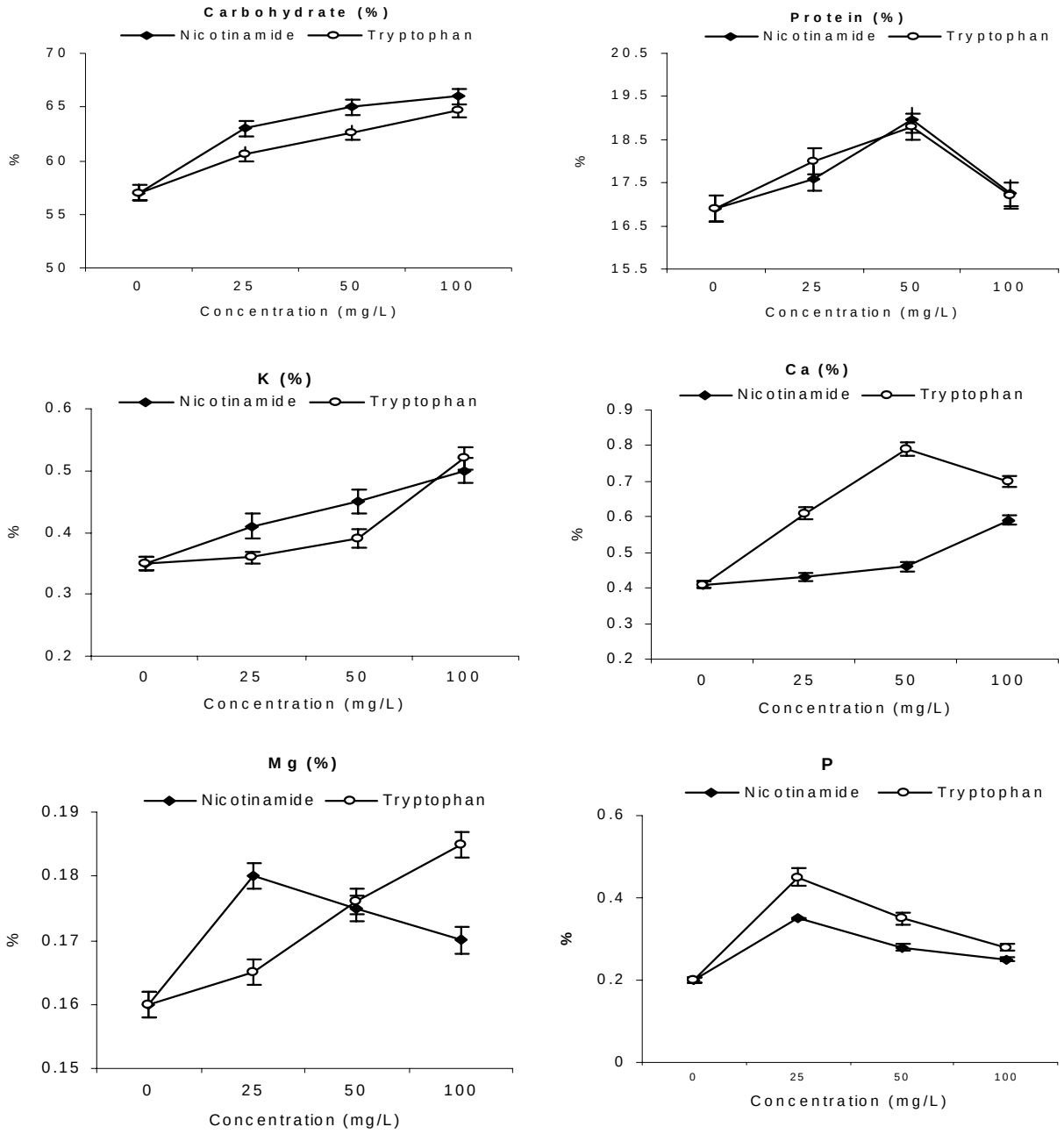
Protein and carbohydrate contents were increased by foliar application of trypyophan, greater increased obtained by 100 mg L^{-1} (Fig. 1). The effect of trypyophan on protein and carbohydrate was previously reported (Landry & Delhaye, 1993; Arshad *et al.,* 1995; Hegazi *et al.,* 1995). Arshad *et al.* (1995) and Ramaih *et al.* (2003) suggested that the effect of tryptophan was due to its conversion to IAA.

The content of K^+, Ca^{2+}, Mg^{2+} and P of grains and shoots significant increased by nicotinamide or tryptophan treatments (Fig. 1, 4). This may be due to the translocation of these elements to the storage organs (grains). Several reports indicate that nicotinamide application increased mineral contents in different crop species (Sharaf EL-Din *et al.,* 1987; Mohamed *et al.,* 1989; Mahgoub *et al.,* 1991).

The increase in the growth and yield parameters of wheat in response to nicotinamide or tryptophan treatments relative to untreated plants might be result from the increased number of tiller, which led to greater number of spike and grains. Increased levels of endogenous IAA, GA_3 and cytokinin in the treated plants (Fig. 2) contributed to enhanced growth and yield, induced by nicotinamide and tryptophan. Plant growth regulators appear either to form a sink mobilizing the different nutrients, which are involved in building new tissues in the wheat plants (Luckwill, 1977) and/or to enhance the photosynthetic mechanism and protein synthesis (Taiz & Zeiger, 1998).

Endogenous phytohormones. Foliar application of nicotinamide or tryptopphan counteracted salinity–induced decline in the concentration of IAA, GA_3 and cytokinin in wheat shoots. Both chemicals reduced the accumulation of ABA as compared with the corresponding untreated plants (Fig. 2). The increase in IAA, GA_3 and cytokinin contents in shoot tissues treated with nicotinamide or tryptophan concurrent with the increase in growth rate (Table II). The data suggests the role of the endogenous hormones in stimulation the cell division and/or cell enlargement and subsequently growth (Wilkins, 1989). Previous published results indicate that exogenous application of nicotinamide leads to IAA, GA_3 and cytokinin accumulation and ABA reduction, which was responsible for changes in growth, development and fruiting of crop plants (Deyab, 1989;

Fig. 1. Effect of different concentrations of nicotinamide or tryptophan on the carbohydrate, protein and minerals percentage (g/ 100 g dry weight) of wheat caryopses. Vertical bars are ± SE.

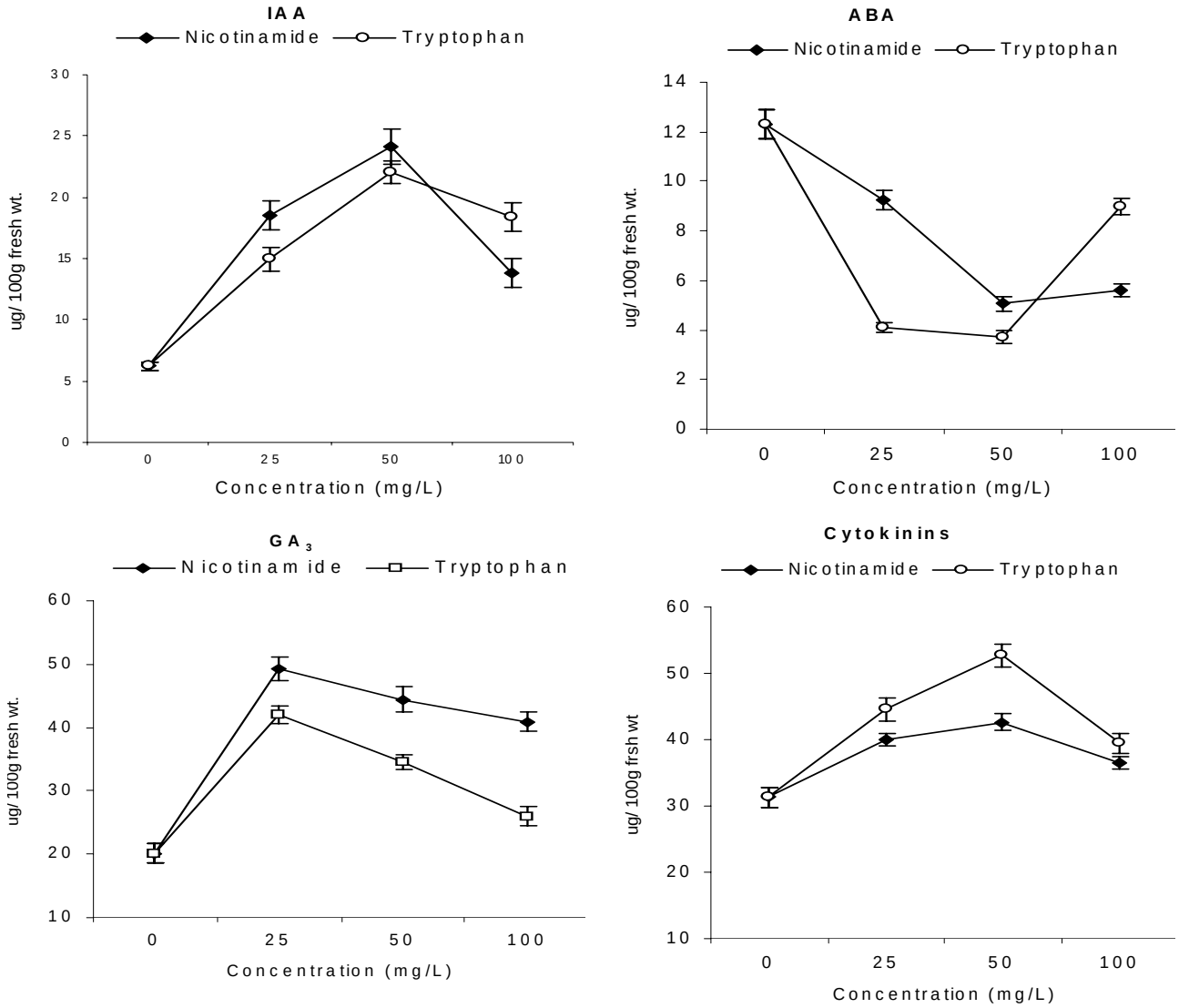


Hathout *et al.*, 1993b). The increase in the IAA in response to tryptophan treatment may be attributed to the involvement of tryptophan in IAA synthesis (Frankenberger *et al.*, 1990; Ramaih *et al.*, 2003).

Photosynthetic pigments. Nicotinamide or tryptophan application to wheat plants significantly increased chlorophyll a and chlorophyll b content, with no significant change in carotenoid content under saline environment (Fig. 3). Similar results have been reported in wheat (Foda, 1987; Sharaf El-Din *et al.*, 1987) and tomato (Hathout *et al.*, 1993a). The effect of nicotinamide on the biosynthesis of chlorophyll may be attributed to its activation of enzymes that regulate photosynthetic carbon reduction (Taylor *et al.*, 1982). Tryptophan induced effect on chlorophyll biosynthesis may be due to its role in IAA biosynthesis (Arshad *et al.*, 1995; Barazani & Friedman, 2000), which was found to lessen the salt-induced decrease in chlorophyll content in *Vigna radiate* (Chakrabarti & Mukherji, 1994).

Inorganic solute composition. Foliar spray of nicotinamide and tryptophan to wheat plants increased the K^+, Ca^{2+}, Mg^{2+}

Fig. 2. Effect of different concentrations of nicotinamide or tryptophan on acidic hormones (IAA, GA_3 and ABA) and cytokinins of shoot apexes. Vertical bars are ± SE.



and P contents whereas decrease the Na^+ and Cl^- contents as compared with the untreated plants (Fig. 4). Foda (1987), Sharaf EL-Din *et al.* (1987) and Mohamed *et al.* (1989) also report an increased level of P, K^+, Ca^{2+} and Mg^{2+} in response to nicotinamide application in different crop plants.

Protein electrophoretic pattern. Wheat shoots treated with nicotinamide or tryptophan indicated qualitative and quantitative differences in protein patterns as compared with plants received only salt imposition (Table III). Wheat plants grown only in salt contained proteins with molecular masses of 216, 124, 90, 57, 37, 32, 28, 23, 17, 15 kDa. HSP 90 was reported to accumulate under salt stress (Pareek *et al.*, 1995). The protein 31-40 kDa seems to be dehydrin (Han *et al.*, 1997). The protein 23 kDa seems to be osmotin, as its expression under salinity was related to increased salt tolerance of tobacco cells (La Rose *et al.*, 1989). Claes *et al.* (1990) found that a 15 kDa protein was induced by salt treated in salt sensitive rice cultivar.

Nicotinamide high concentrations (50 & 100 mg L^{-1}) decreased the number of protein to eight. In addition, some proteins disappeared whereas others were accumulated after nicotinamide application (Table III). The intensity of salt response protein 57, 37, 23 and 17 kDa detected in wheat shoots were increased by nicotinamide foliar spraying (Table III). Nicotinamide may have a role in various stress through increasing the frequency of DNA single strand breaks (Berglund, 1994), and activating the transcription of defense genes (Suzuki *et al.*, 1995).

Foliar application of tryptophan increased the number of polypeptide relative to untreated plants (Table III). New set of proteins consisted of 3, 5 and 5 polypeptides were appeared with 25, 50 and 100 mg L^{-1} tryptophan application, respectively (Table III). Small molecular masses (28, 23 & 17 kDa) were increased by tryptophan foliar spraying to wheat under salinity. Similarly, dehydration induced 23 kDa in barley and 17 kDa in maize

Table I. Mechanical and chemical analysis of soil of Abo Mady region (North Delta) where the experiments were carrier out

Mechanical analysis											pH	EC (dSm^{-1})
Soil fraction (%)											8.13	10.8
Sand		Silt			Clay			Texture class				
17.9		38.3			43.9			Clay				
Chemical analysis												
$CaCO_3$ (%)	Organic mater (%)	Cations (meq/L)				Anions (meq/L)				Available nutrient (ppm)		
2.13	0.74	Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	N	P	K
		4.33	1.14	13.10	16.60	-	1.12	35.6	0.98	23.9	4.7	399.5

Table II. Effect of foliar application of nicotinamide or tryptophan on the growth parameters (measures after 75 d) and yield components (measured after 160 d) of wheat plants (average of two seasons)

Treatment		Plant height (cm)	Tillers number (m^2)	Area of leaves (cm^2)/tiller	Dry weight of tillers (g/m^2)	Grain weight (g/m^2)	1000-grain weight (g)	Grain Yield (Kg/fed)	Straw yield (Kg/fed)	Crop index	Harvest index
Untreated plants		72.00	476	50.29	688	350	37.56	1368	2615	54.39	35.23
Nic.	25	76.20	516	65.25	912	416	43.74	1739	3236	57.27	36.42
(mg/L)	50	77.38	544	68.27	1004	458	45.14	2024	3493	60.00	37.50
	100	76.20	531	66.88	979	444	44.76	1865	3415	57.10	36.34
Try.	25	75.40	605	74.72	1049	437	42.15	1836	3176	57.81	36.60
(mg/L)	50	73.00	583	70.85	977	488	42.89	2033	3616	59.88	37.32
	100	74.80	571	67.90	897	469	41.84	1970	3343	60.22	37.79
LSD 5%		5.07	34.77	3.84	74.18	30.27	2.17	95.40	106.36	3.46	N.S
LSD 1%		7.12	48.80	5.39	104.13	42.49	3.05	133.91	149.29	4.85	N.S

N.S., non significant Nic., nicotinamide Try., tryptophan

Table III. Relative area (%) of each band of the comassie blue–stained gels of wheat shoots treated with different concentration of nicotinamide or tryptophan in presence of salt stress

Band number	Mwt KDa	Untreated Plants	Nicotinamide mg/L			Tryptophan mg/L		
			25	50	100	25	50	100
1	216.4	18.8	14.6	18.1	14.2	13.4	13.5	7.6
2	168.4	-	-	-	-	-	9.7*	9.2*
3	153.5	-	11.6*	7.4*	11.1*	6.6*	-	5.4*
4	123.5	7.7	-	-	-	-	-	-
5	105.4	-	-	-	-	6.9*	7.1*	5.2*
6	90.3	6.0	-	-	-	7.1	-	-
7	83.2	-	7.0*	-	-	-	-	-
8	65.2	-	-	-	-	-	18.8*	13.6*
9	57.1	25.2	29.0	35.9	22.7	26.5	-	12.8
10	42.1	__	-	-	-	3.6*	4.6*	-
11	39.3	__	-	-	-	-	15.2*	16.5*
12	36.8	5.4	7.7	6.5	10.6	10.0	3.8	-
13	32.4	5.7	3.6	-	-	5.5	-	-
14	28.4	4.8	5.3	5.9	5.2	5.5	5.9	5.1
15	22.8	3.5	4.3	3.8	10.6	4.7	4.4	5.3
16	17.3	5.2	7.0	9.8	14.4	4.0	5.3	9.9
17	15.0	17.7	9.9	12.6	11.2	6.2	11.7	9.4
Total number of band		10	10	8	8	12	11	11
Number of new responsive protein (*)			2	1	1	3	5	5

(Close *et al.*, 1989). Since tryptophan could be a precursor of IAA (Ramaih *et al.*, 2003), which was reported to increase amino acid incorporation into proteins in soybean (Morre *et al.*, 1984), it is proposed that tryptophan may acts in the same way as noted in this study.

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Fig. 3. Effect of different concentrations of nicotinamide or tryptophan on Chlorophyll a, Chlorophyll b and carotenoids of wheat leaves. Vertical bars are ± SE.

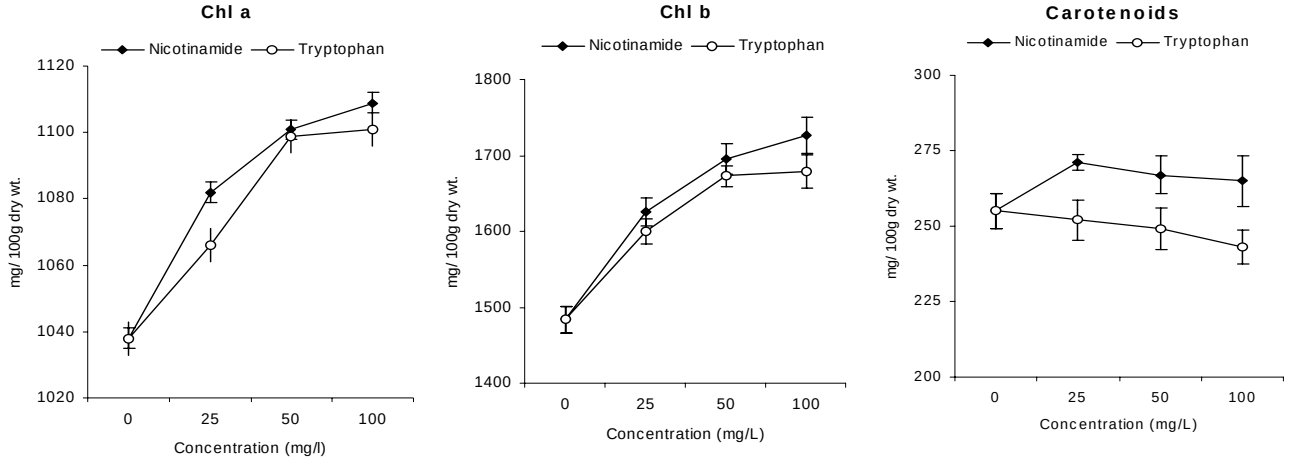
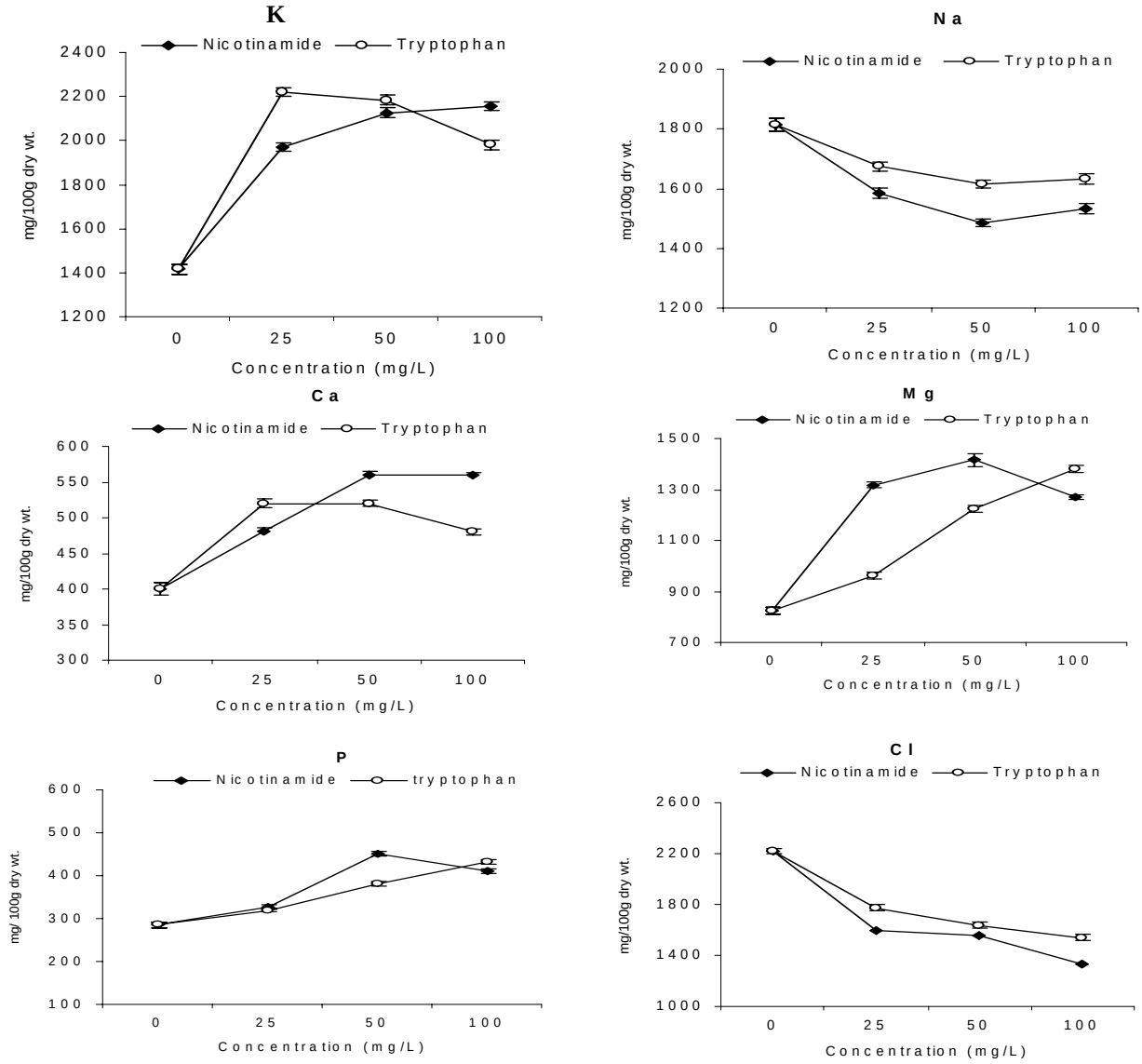


Fig. 4. Effect of different concentrations of nicotinamide or tryptophan on the ionic contents of wheat shoots. Vertical bars are ± SE.



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(Received 15 February 2005; Accepted 14 May 2005)