FLUORIDE-INDUCED CHANGES IN CARBON AND NITROGEN METABOLISM IN TWO CONTRASTING CULTIVARS OF *TRITICUM AESTIVUM* L.

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ABSTRACT: The effect of 100–200 ppm sodium fluoride (NaF) on the activities of sucrose catabolizing enzymes, amylase, and transaminases in relation to the transformation of starch to free sugars and soluble protein in the root and shoot of two wheat (*Triticum aestivum* L.) cultivars, HD 3086 and WH 1105, was studied on the 7th day of germination. Application of the fluoride ion (F) drastically reduced starch content and caused a marked accumulation of reducing and non-reducing sugars in the root and shoot indicating their protective role during stress conditions. F inhibited the activities of soluble acid invertase, neutral invertase, and amylase. Higher acid invertase activity over neutral invertase indicates its predominant role in sucrose hydrolysis. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities also increased with F addition in correspondence with an increase in the soluble protein and amino acid contents. Apparently, the *T. aestivum* seedlings respond to F-mediated disruption of carbon metabolism by a compensatory effect on nitrogen metabolism.

Keywords: Amino acid; Enzyme activities; Fluoride; Protein; Starch; Sugar; *Triticum aestivum* L.

INTRODUCTION

The fluoride ion (F) occurs naturally in the groundwater in varying amounts in many states of India.[1] The presence of F in higher concentrations in groundwater is highly toxic for plants[2, 3] and thereby for animals[4-6] and human beings.[7-9] High F concentrations, particularly above 100 ppm, decrease biomass production and crop productivity.[10] F inhibits starch biosynthesis in tubers of *Solanum tuberosum* (potato) and grains of *Sorghum bicolor* (jowar) but its effects on sucrose biosynthesis are either inhibitory or promotory.[11-13] F often inhibits enzymes that require cofactors such as Ca^{2+}, Mg^{2+}, and Mn^{2+} ions. The inhibition of amylase and invertase activities can be attributed, in part, to removal of the Ca^{2+}, Mg^{2+}, and Mn^{2+} cofactors. F inhibits amylase and invertase activities in germinating *Vigna radiata* seedlings[1]. However, F tolerant plants possess a better expression of carbohydrate and nitrogen metabolism.

Aminotransferases such as glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are responsible for the biosynthesis of glutamine to other amino acids and thus play an important role in regulation of nitrogen metabolism in crop plants.[15] Enzymes of nitrogen metabolism are often up-regulated by an elevated sugar content in plant tissues.[16] Increase in soluble protein in pollen of *Oryza sativa* (rice) under high temperature stress was also observed[17] which contributes to the maintenance of cell structure and function under abiotic stresses.

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Wheat (*Triticum aestivum* L.) is an important cereal, whose productivity depends upon the production, translocation, and utilization of carbohydrates. It accounts for the majority of food products used for human diets. Wheat is particularly sensitive to F toxicity as F is readily absorbed by its roots and translocated to other parts of plants where cellular metabolism is drastically affected.[18] Even though much supporting evidence on the role of sugars against F toxicity is available,[11, 12] information on the enzymes involved in carbon and nitrogen metabolism under F stress is very limited. Therefore, in the present study, we have investigated the changes in carbon and nitrogen status in the forms of soluble sugars, amino acids, and proteins, and the activities of the carbohydrate metabolising enzymes and aminotransferases in the F-treated and non-treated seedlings of *Triticum aestivum* cultivars.

MATERIALS AND METHODS

PLANT MATERIAL: Seeds of two *Triticum aestivum* L. cultivars, HD 3086 and WH 1105, were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The seeds were surface sterilized with mercuric chloride (0.1%) for 1 min and rinsed thrice with distilled water. One set of these cultivars was grown in petri plates (10 seeds for each cultivar) on germination paper moistened with 4 mL of distilled water at 25±1ºC in continuous dark conditions. Two concentrations of NaF, 100 and 200 ppm, were chosen based on the different responses to them in growth characteristics. Uniform sized wheat seedlings were sampled on the 7thday of germination. All measurements were performed on root and shoot in triplicate.

BIOCHEMICAL PARAMETERS:

Carbohydrates: Total sugar, reducing sugar, and starch in the shoot and root were extracted and estimated according to the procedure as described earlier.[19] Starch was extracted with perchloric acid and estimated from the sugar free residue by multiplying the amount of glucose by 0.9.[1] The concentration of non-reducing sugar was calculated by subtracting reducing sugar from total sugar.

Invertase and amylase: The soluble invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) activity, glutamate oxaloacetate transaminase (EC 2.6.1.1), glutamate pyruvate transaminase (EC 2.6.1.2), content of protein, and amino acid in root and shoot were determined as previously described.[20] Amylase (EC 3.2.1.1) activity was also extracted and assayed.[21]

RESULTS

In the present investigation, seedlings of two *T. aestivum* cultivars, HD 3086 and WH 1105, were cultured for 7 days in the absence (control) and presence of F (100 and 200 ppm NaF) for studying carbon and nitrogen metabolism in root and shoot and the results obtained are presented in Figures 1, 2, and 3.

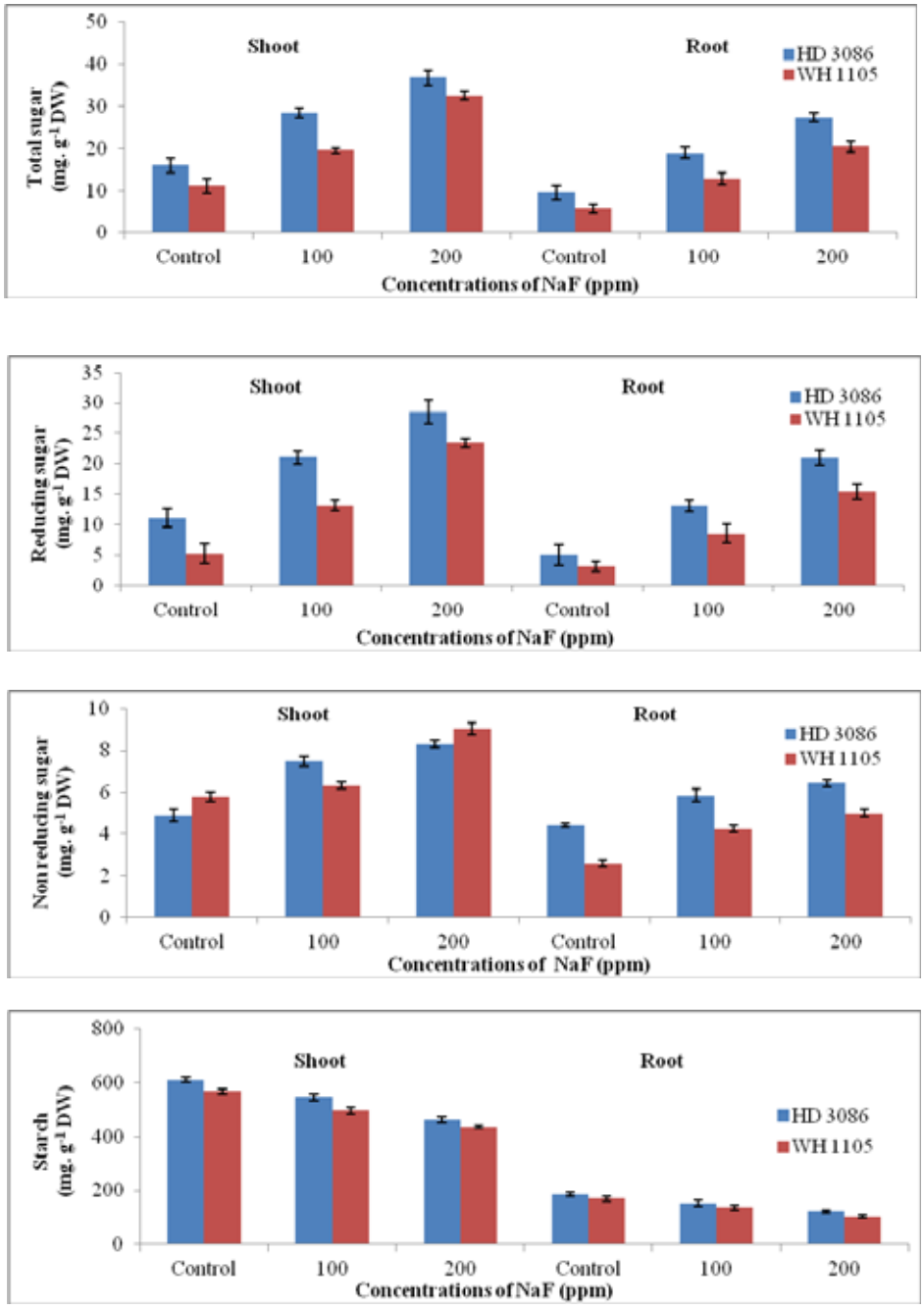

Figure 1. Effect of NaF on contents of total sugar, reducing sugar, non-reducing sugar, and starch in shoot and root of *T. aestivum* on the 7th day of germination. Total sugar in shoot: G=1.45, T=1.78, G×T=2.52; total sugar in root: G=1.32, T=1.62, G×T=2.41; reducing sugar in shoot: G=1.37, T=1.68, G×T=2.94; reducing sugar in root: G=1.33, T=1.63, G×T=2.75; non-reducing sugar in shoot: G=NS, T=0.29, G×T=0.41; non-reducing sugar in root: G=0.19, T=0.24, G×T=0.57; starch in shoot: G=6.77, T=8.29, G×T=2.75; and starch in root: G=5.11, T=6.23, G×T=NS, where G is genotype, T is treatment and G×T is the genotype and treatment interaction. The values of G, T, and G×T are the critical differences at the 5% level of difference while the vertical bars on the graphs represent the standard errors. NS=not significant.

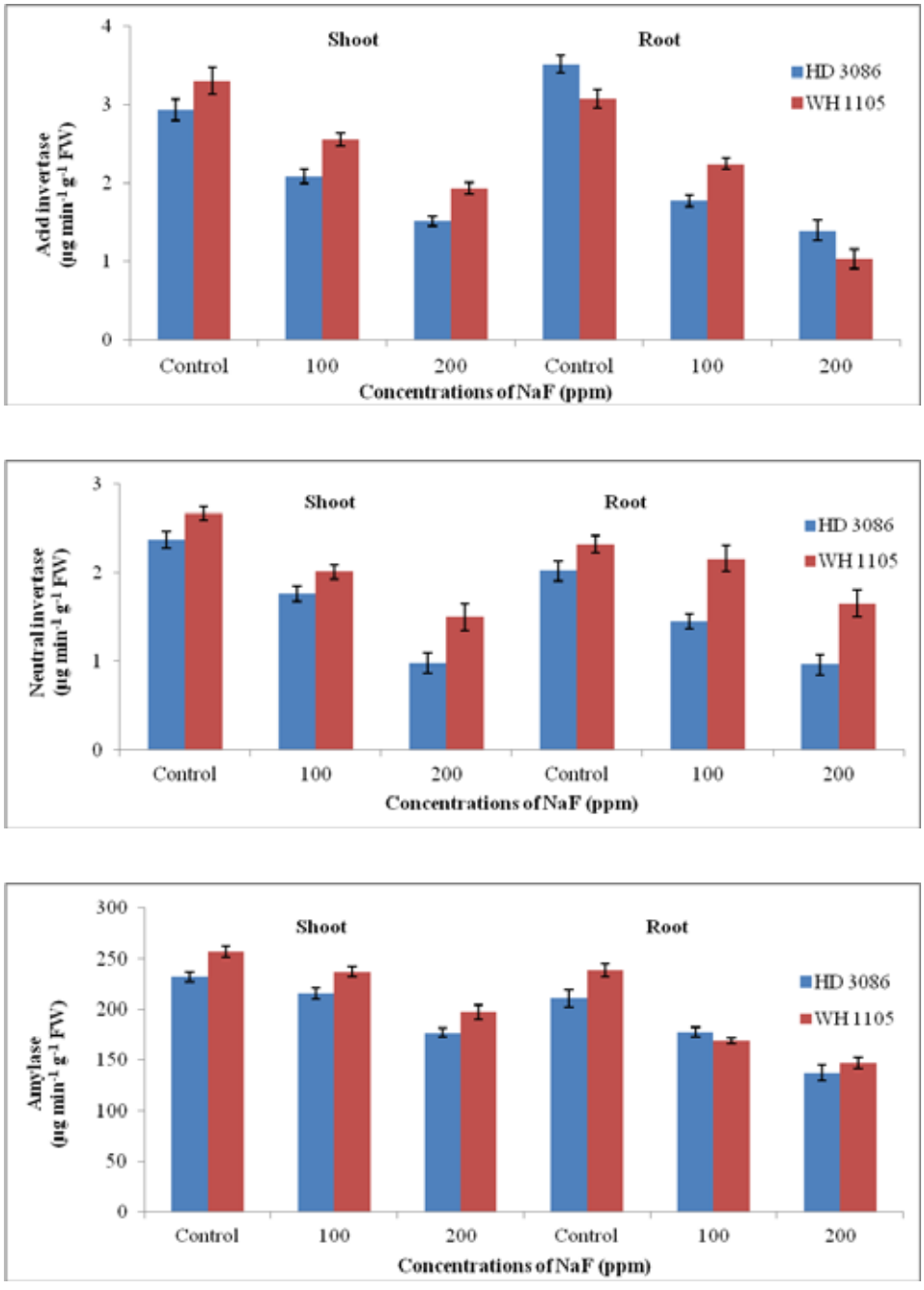

Figure 2. Effect of NaF on activities of acid invertase, neutral invertase, and amylase in shoot and root of *T. aestivum* on the 7th day of germination. Acid invertase in shoot: G=0.11, T=0.13, G×T=0.16; acid invertase in root: G=0.11, T=0.13, G×T=0.19; neutral invertase in shoot: G=0.10, T=0.21, G×T=NS; neutral invertase in root: G=.012, T=0.15, G×T=0.21; amylase in shoot: G=5.73, T=7.02, G×T=NS; amylase in root: G=6.47, T=7.93, G×T=11.22; where G is genotype, T is treatment and GXT is genotype and treatment interaction. The values of G, T, and G×T are the critical differences at the 5% level of significance while the vertical bars on the graph represent the standard errors. NS=not significant.

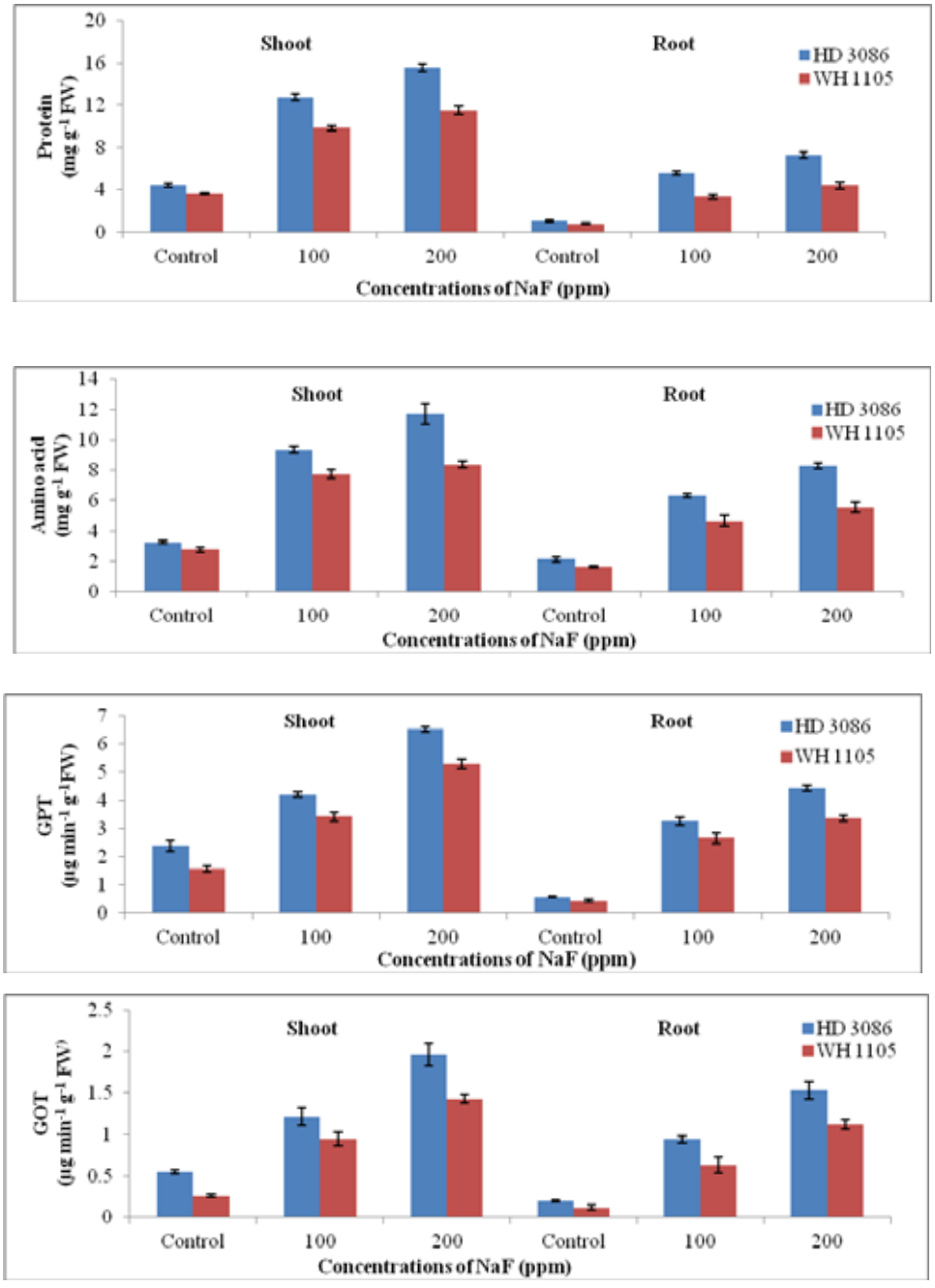

Figure 3. Effect of NaF on contents of protein, amino acid, and activities of GPT and GOT in shoot and root of *T. aestivum* on the 7th day of germination. Protein in shoot: G=0.28, T=0.34, G×T=0.48; protein in root: G=0.21, T=0.26, G×T=0.37); amino acid in shoot: G=0.35, T=0.42, G×T=0.60; amino acid in root: G=0.25, T=0.31, G×T=0.43; GPT in shoot: G=0.10, T=0.18, G×T=0.25; GPT in root: G=0.12, T=0.14, G×T=0.20; GOT in shoot: G=0.83, T=0.10, G×T=0.14; and GOT in root: G=0.68, T=0.84, G×T=0.12, where G is genotype, T is treatment, and GXT is genotype and treatment interaction. The values of G and G×T are the critical differences at the 5% level of significance while the vertical bars on the graph represent the standard errors.

Increasing the concentration of F from 100 to 200 ppm resulted in a significant increase in the contents of total free sugar, reducing sugar, and non-reducing sugar while the starch content was decreased in root and shoot of both the cultivars as compared to control (Figure 1). Shoot had a higher content of reducing sugars, non-reducing sugar, and starch as compared to root. Sucrolytic enzymes such as acid invertase and neutral invertase and the total amylase activity revealed their decreasing trend with increasing F concentrations in the seedlings (Figure 2). The activity of acid invertase predominated over neutral invertase in both root and shoot in the control and the F-stressed seedlings. However, aminotransferase (GOT and GPT) activity increased in parallel with an increasing content of soluble protein and amino acid under F treatment in comparison to control (Figure 3). In general, when compared to WH 1105, HD 3086 showed higher activities of GOT and GPT along with higher contents of total sugar, reducing sugar, starch, protein, and amino acid. On the other hand, WH 1105 revealed higher acid invertase, neutral invertase, and amylase activities along with an increased content of non-reducing sugar.

In both the control and F treatment groups there was a significant correlation between sugars and amino acids ($r=0.972$, $p\leq0.01$; $r=0.942$, $p\leq0.01$, respectively) and between protein and both GOT and GPT ($p\leq0.01$). With F treatment, there was (i) a positive correlation ($r=0.742$; $p\leq0.01$) between sugars and acid invertase; (ii) a negative correlation between sugars and neutral invertase ($r= –0.564$; $p\leq0.01$); (iii) a positive correlation between starch and amylase activity ($r=0.914$; $p\leq0.01$) and (iv) a stronger correlation between protein and GPT ($r=0.995$; $p\leq0.01$) than between protein and GOT ($r=0.886$; $p\leq0.01$) (Table).

Table. Correlation coefficients between acid invertase, neutral invertase, amylase, amino acids, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and the content of sugars, starch, and protein in the control and fluoride groups

	Acid invertase		Neutral invertase		Amylase	
	Control	Fluoride	Control	Fluoride	Control	Fluoride
Sugars	–0.344	0.742*	0.230	–0.564*		
Starch					0.548	0.914*
	Amino acids		GOT		GPT	
	Control	Fluoride	Control	Fluoride	Control	Fluoride
Sugars	0.972*	0.942*				
Protein			0.870*	0.886*	0.983*	0.995*

*$p\leq0.01$.

DISCUSSION

Starch, the major storage product of *T. aestivum* L. is hydrolysed by amylases to glucose during germination. With the progress of seedling growth, the content of total free sugar, reducing sugar, and non-reducing sugar increased tremendously with the addition of F. F application increased the mobilisation of starch as indicated by the lower starch content and reduced activity of amylase in both root and shoot. A similar reduction in starch content of the *Sorghum vulgare* has been reported.[12] F-induced stimulation of starch breakdown in plant organs was accompanied by increased synthesis of sucrose.[22,23] Sugar accumulation in the form of sucrose is probably associated with the balance between the breakdown and synthesis reactions that is governed by several enzymes. So, we can infer that non-reducing sugar accumulation in form of sucrose is attributable to the difference in the activities of both acid invertase and neutral invertase. Nevertheless, the higher acid invertase activity in the shoot appeared to be responsible for generating reducing sugars for the growth processes and stress tolerance. Neutral invertase activity, although affected by F stress was low as compared to acid invertase activity. A major perturbation of plant protection under F stress is ascribed to carbon metabolism which is manifested in degradation of starch to soluble sugars and this rate is strictly dependent on the F concentration.[24,2] The considerably higher accumulation of sugars and soluble protein, and the increased aminotransferase activities in HD 3086, compared to WH 1105, makes this genotype more tolerant to F. In fact, the supply of assimilable sugar and soluble protein was not responsible for the decline in growth of the seedlings under F stress and some other metabolic processes is likely to be involved in the growth decline.[26] Breakdown of starch by amylase further indicated that stress had caused stimulation of starch degradation to generate sugars to overcome F stress. Accumulation of soluble sugars helps in osmotic adjustment, membrane protection, and stimulating activities of proteins and enzymes.[18,27] Sucrose and starch metabolism are both strongly influenced by F as the activities of both invertase and amylase decreased with increasing F concentrations. In general, amylase has been implicated either in the breakdown of starch for the production of energy through metabolism of its released glucose monomer and/or generation of primer molecules for starch synthesis.[28,29]

Interestingly, the soluble protein and amino acid contents increased with F addition. Likewise, the activities of the two transaminases, i.e., GOT and GPT, also increased. F apparently causes a shift in the balance of carbon and nitrogen metabolism by stimulating starch degradation and funnelling the accumulating sugars into respiratory metabolism and thus generating carbon skeletons for amino acid biosynthesis. An increase in the F concentration has resulted in a significant increase in the amino acid content in *Oleae uropaea* L. (olive).[30] Proteins, being one of the important organic nitrogenous constituents of plants, play a vital role in the compensatory metabolism of a plant species during F stress conditions. The increase in protein content may be due to a synergistic effect of F with nitrogen as protein synthesis is directly related to the nitrogen concentration.[31,32] GOT and GPT are the key aminotransferase enzymes, which can promote amino acid

decomposition as well as the synthesis of new amino acids. GOT and GPT are mainly responsible for supplying N in the form of amino acids and proteins to the developing plant organs.[33] Since the activity of transaminases is stimulated under stress conditions, these enzymes may possibly be acting in the direction of deamination to provide amino acids, especially glutamine, for the common N-pool.[34] Therefore, it may be interpreted that the resulting amino acids were substrates for GOT and GPT and thus inducing these enzymes activities for nitrate assimilation, translocation, and reassimilation between the source and the sink.

CONCLUSIONS

F apparently causes a shift in the balance of carbon and nitrogen metabolism by inhibiting starch biosynthesis and funnelling the accumulating sugars into respiratory metabolism and thus generating carbon skeletons for amino acid biosynthesis. Cultivar HD 3086 seemed to respond better under F toxic effects than WH 1105 due to higher GOT and GPT activities and accumulation of sugars and soluble protein which can function as an osmolyte to maintain cell turgor and thus help in protecting membrane and protein.

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