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Apical Stem Culturing to Enhance Cell Sap Assimilates towards Grain Sucrose and Glutamine Metabolism in Wheat

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Apical stem culturing offers an alternative approach of manipulating cell sap for wheat grain carbon and nitrogen metabolism in a near *in vivo* conditions. Employing this technique, role of sucrose and glutamine in transport stream on sucrose metabolism, ammonia assimilation and aminotransferase activities were assessed towards starch and protein accumulation in two wheat genotypes PBW 343 (low yield) and PBW 621 (high yield). At mid-milky stage, detached tillers were cultured in complete liquid medium containing varied concentration of glutamine and sucrose for seven days during year 2012–13 and 2013–14. Increasing glutamine concentration from 17 to 25 mM in the culture medium having 117 mM sucrose enhanced activities of nitrate reductase, glutamate synthase, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) at 4 days after culturing (DAC) in correspondence with an increase in soluble protein content. However, at 7 DAC content of soluble protein decreased whereas starch accumulation increased showing, thereby a compensatory effect on carbon and nitrogen metabolism. Apparently, activities of sucrose synthase, soluble acid and neutral invertase significantly decreased. Increasing sucrose concentration from 117 to 125 mM led to an increase in transformation of sugars to starch in grain but protein content decreased. PBW 621 showed high protein content due to higher activities of GOT, GPT at 4 DAC which subsequently increased carbon skeleton of proteins towards starch synthesis at 7 DAC. Grain filling processes in terms of soluble sugars/starch were strongly correlated to invertase activities whereas proteins to aminotransferases.

Keywords: acid invertase, carbon/nitrogen metabolism, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, sucrose synthase

Abbreviations: NR – nitrate reductase, GOGAT – glutamate synthase, GOT – glutamate oxaloacetate transaminase, GPT – glutamate pyruvate transaminase, SS – sucrose synthase, AI – acid invertase, NI – neutral invertase, DAC – days after culturing

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Introduction

Despite numerous studies on grain carbon and nitrogen metabolism in wheat, information regarding manipulation of grain filling processes in near *in vivo* conditions is scanty. Tissue culture technique provides an alternative approach for studying grain metabolism by apical stem culturing technique in near *in vivo* conditions. Studies conducted in the past indicated that much of the increasing dose of nitrogen fertilizer supplied in soil results in tremendous losses of nitrogen to the atmosphere due to denitrification and leaching (Luo et al. 2006; Anjana et al. 2007). Moreover, the efficiency of uptake and utilization of nitrogen also varied within genotypes. During endosperm development, the processes of starch and protein accumulation is largely determined by the assimilate entering from vegetative tissues into the grain. Therefore, increasing nitrogen supply to the apical stem in culture medium not only increases grain nitrogen content (Barlow et al. 1983; Paul and Foyer 2001) but also influences the balance of carbon and nitrogen in the endosperm (Paul and Pellny 2003). Manipulation of sucrose and nitrogen in the sap entering seed is an effective way for regulating the seed composition with respect to protein and starch contents in wheat (Koch 2004).

The physiology of grain development is complex and far from being clear because of the difficulty of regulating carbon and nitrogen applications to grains (Zhou et al. 2006). Several key enzymes involved in the synthesis of starch and protein involves nitrate reductase (NR) and glutamate synthase (GOGAT) that play an important role in nitrate to glutamate conversion (Morozkina and Zvyagilskaya 2007; Masclaux-Daubresse et al. 2010). Sucrose synthase (SS), acid invertase (AI) and neutral invertase (NI) are key enzymes regulating metabolism of sucrose and its conversion ability to starch (Jiang et al. 2003). Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are responsible for the biosynthesis of glutamine to other amino acids, forming storage protein in grain and thus play an important role in regulation of N metabolism in crop plants (Lea et al. 1990).

In this study, the effect of increasing concentration of sucrose and glutamine to detached apical stems carrying ear heads at mid milky stage were studied for accumulation of starch, protein, amino acids alongwith activities of enzymes involved in sucrose metabolism, ammonia assimilation and aminotransferase in wheat grains.

Materials and Methods

Plant material and growth condition

Two wheat (*Triticum aestivum* L.) genotypes, namely PBW 343 and PBW 621 were selected for tiller culturing experiment during year 2012–13 and 2013–14. Ears at mid-milky stage, i.e. 12–15 days post anthesis (DPA) were cut under water below penultimate node and cultured according to the method of Asthir and Bhatia (2014) keeping twelve replications for each treatment. To study the response of these cultivars to different glutamine and sucrose doses, culture media having four treatments: suboptimal N dose (5 mM glutamine), optimal N dose (17 mM glutamine), supra-optimal N dose (25 mM

glutamine) at optimal sucrose concentration (117 mM) and to study the effect of higher sucrose concentration from 117 to 125 mM on carbon and nitrogen metabolism supra-optimal sucrose (125 mM) at optimal N dose (17 mM glutamine) were used. After adjusting the pH of the culture solution to 5.5, the medium was ultra filtered through 0.22 μM millipore membrane. Before culturing, the flag leaf and its sheath were removed and stems were surface sterilized with 40% ethanol followed by quick washing with distilled water. Ear-heads carrying 20 cm peduncle length from the cut end were placed (one ear-head per tube) in culture tube containing 35 ml cold-sterilized liquid medium. These cultured ear-heads were then transferred to water bath maintained at 2–4 °C in the natural day light conditions. The grains at 4 days and 7 days after culturing were separated and used for analysis.

Extraction and assay of enzymes

Nitrate reductase

The nitrate reductase (NR) activity was estimated by the method described by Jaworski (1971). Developing grains (200 mg) were homogenized in 5 ml of 0.1 M phosphate buffer (pH 7.5) containing 0.05 ml *n*-propanol and 0.25 g KNO_3 and incubated in a metabolic shaker at 30 ºC in dark for 90 min. The release of nitrite (NO_2^-) into the medium was determined by treating 1 ml of aliquot with 1 ml of 1% sulphanilamide in 1M HCl and 0.02% N-(1-napthyl) ethylene diamine dihydrochloride (NEDD) and the volume was made to 10 ml with distilled water and kept in darkness for 15 min. After 20 min, the pink color developed was recorded at 540 nm using Spectronic-20. The standard curve was prepared using 0–10 μg of KNO_2. NR activity was expressed as μmol NO_2^- formed h^{-1} g^{-1} FW.

Glutamate synthase

Glutamate synthase (GOGAT) activity was extracted by the method of Mohanty and Fletcher (1980). Developing grains (1 g) was homogenized in liquid nitrogen with 5 ml of extraction medium containing 100 mM Tris-HCl (pH 7.5), 0.2 M sucrose, 10 mM KCl, 10 mM $MgCl_2$, 10 mM EDTA and 10 mM β-mercaptoethanol was added. Extract was filtered through muslin cloth and centrifuged at 10,000 *g* for 30 min at 4 °C. Reaction mixture (3 ml) contained 75 μmol Tris-HCl buffer, 10 μmol α-ketoglutarate, 15 μmol L-glutamine, 0.3 μmol NADH and enzyme extract. Reaction was initiated by addition of NADH. Enzyme kinetics was studied for 3 min at 340 nm and expressed as μmol NADH oxidized min^{-1} g^{-1} FW.

Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity

Glutamate oxaloacetate transaminase (GOT) was assayed according to Tonhazy (1960a). A 0.2 ml of 0.1 M 2-oxoglutarate solution was added to a mixture of 0.5 ml of 0.1 M L-aspartate in 0.1 M potassium phosphate buffer (pH 7.5), 0.1 ml 2 mM pyridoxal phosphate and 0.2 ml of enzyme extract. The incubation was done at 37 °C for 10 min. The reaction was terminated by the addition of 0.1 ml of TCA solution, shaken vigorously and then 0.2 ml of aniline citrate was added. The contents were mixed and allowed to stand for

10 min. Pyruvate produced, together with excess of 2-oxoglutrate was converted to their hydrazones with the addition of 1 ml of chromogen solution (5 mM 2,4-dinitrophenyl hydrazine). The contents were shaken with 2 ml of water saturated toluene and then centrifuged. One ml of upper layer was drawn into another test tube and to this 5 ml of alcoholic KOH was added. After 5 min, 1 ml of distilled water was added and the colored solution was measured at 520 nm and the enzyme activity was calculated from the standard curve using oxaloacetate (0.3–1.8 μmol).

For assaying the activity of glutamate pyruvate transaminase (GPT), the same procedure employed for GOT was followed except that aniline citrate step was omitted (Tonhazy 1960b). The absorbance of colored solution containing pyruvate hydrazone, selectively extracted with water saturated toluene, was measured at 520 nm. For enzyme activity, the amount of pyruvate formed was calculated from the curve of authentic pyruvate standards (0.3–1.8 μmol) run simultaneously.

Soluble acid invertase, soluble neutral invertase and sucrose synthase

Grain samples (1 g) were homogenized at 0°C in 50 mM Hepes-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM sodium EDTA, 2.5 mM DTT, 0.5 mg ml^{-1} BSA and 0.05% (v/v) Triton X100 by the method of Stommel (1992). Homogenates were centrifuged at 10,000 *g* for 15 min at 0°C. The pellet was a resuspended in extraction buffer and centrifuged as before. The pellet was dissolved in minimum volume of extraction buffer and dialysed overnight against four times diluted extraction buffer without $MgCl_2$, EDTA, DTT and BSA. The activity of sucrose synthase (UDPglucose-fructose-2-α-D glucosyltransferase) was assayed by the method of Morell and Copeland (1985). The soluble acid invertase (pH 4.8) and soluble neutral invertase (pH 7.5) (β-D-fructofuranoside fructohydrolase) activities were assayed essentially by the method described previously by Singh and Asthir (1988).

Extraction and estimation of various metabolites

Free sugars and starch

Free sugars were extracted sequentially with 80% and 70% ethanol and the extracts were concentrated by evaporating off the ethanol under vacuum. The total free sugars were determined colorimetrically using the reaction with phenol (Dubois et al. 1951). From the sugar free residue so obtained, starch was extracted with cold (2–4 °C) perchloric (52%), purified with iodine precipitation and estimated as described by Yoshida et al. (1976).

Soluble protein, amino acid and nitrogen content

Soluble proteins were extracted in 0.1 M NaOH and precipitated with trichloroacetic acid and estimated by the method of Lowry et al. (1951). The amino acid was estimated by method described by Lee and Takahashi (1966) and nitrogen content was estimated by the method of McKenzie and Wallace (1954).

Statistical analysis

All the values reported in this paper were the mean of three replicates. All data obtained was subjected to analysis of factorial experiment in CRD at 5% level of CD. In all the tables ± values representing standard error of the means.

Results

In this study, detached apical stem cultured for 4 and 7 days in presence of varied concentration of glutamine under optimal (Control, 17 mM), suboptimal (5 mM), supraoptimal (25 mM) doses and sucrose under optimal (117 mM), supraoptimal (125 mM) doses were analysed for grain carbon and nitrogen metabolism of two wheat genotypes PBW 621 (high yielding) and PBW 343 (low yielding) and the results obtained are presented in Tables 1, 2, 3 and 4.

Effects of glutamine concentration on grain carbon and nitrogen metabolism

Increasing concentration of glutamine from 5–25 mM (T1–T2) resulted in a significant increase in activities of nitrate reductase (NR), glutamate synthase (GOGAT), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in parallel with an increasing content of soluble protein and nitrogen at 4 DAC as compared to control (C) conditions (Tables 1 and 2). In contrast, the activities of sucrose synthase (SS), acid invertase (AI) and neural invertase (NI) in grains rapidly decreased, which results in decreasing sugars and starch content at 4 DAC. Whereas the activities of NI increased along with increasing content of amino acid and starch at 7 DAC. In contrast, with decreasing glutamine concentration from 17–5 mM (C-T1) showed decrease in NR, GOGAT, GOT and GPT activity.

Effects of sucrose concentration on grain carbon and nitrogen metabolism

With increasing sucrose concentrations from 117–125 mM (C-T3) in culture medium, the contents of protein, amino acid and nitrogen in grains decreased alongwith decreasing activities of NR and GOGAT, whereas slight increase in activities of GOT and GPT were observed (Tables 1 and 2). However, the activities of SS (synthesis), AI and NI were increased significantly resulting in increasing sugar (4 DAC) and starch content (7 DAC) (Tables 3 and 4).

In general, PBW 621 showed higher activities of NR, GS, GOGAT, GOT, GPT and AI in concomitant with increased content of soluble protein, nitrogen at 4 DAC and amino acid, starch at 7 DAC (Tables 1, 2, 3 and 4). On the other hand, PBW 343 revealed higher SS activity along with increased sugar content.

A significant correlation of GOGAT, GOT and GPT with protein content $r = 0.64, 0.96, 0.93$, respectively ($p \leq 0.01$) was observed (Table 5). Nitrate reductase is positively correlated with GOGAT $r = 0.71$ ($p \leq 0.01$); GOGAT with GOT and GPT $r = 0.69, 0.75$, respectively ($p \leq 0.01$); sucrose synthase with acid invertase $r = 0.80$ ($p \leq 0.01$). However, SS, AI and NI were positively correlated with sugar content $r = 0.49, 0.45, 0.49$,

Table 1. Effect of glutamine and sucrose at different levels on nitrate reductase (NR, µmol NO_2^- released /h/g FW), glutamate synthase (GOGAT, µmol NADH oxidized/min/g FW), glutamate oxaloacetate transaminase (GOT, µmol oxaloacetate min^{-1} g^{-1} FW) and glutamate pyruvate transaminase (GPT, µmol pyruvate min^{-1} g^{-1} FW) activities in grains of two wheat genotypes (PBW 343, PBW 621) at 4 and 7 days after culturing

Genotypes \ Enzymes		NR		GOGAT		GOT		GPT	
		4	7	4	7	4	7	4	7
PBW 343	C	0.71 ± 0.03	0.56±0.07	0.76 ± 0.07	0.55 ± 0.16	8.82 ± 0.08	2.26 ± 0.07	3.24 ± 0.07	1.13 ± 0.03
	T1	0.39 ± 0.02	0.15 ± 0.06	0.29 ± 0.05	0.16 ± 0.06	7.79 ± 0.04	1.13 ± 0.19	2.04 ± 0.09	0.54 ± 0.09
	T2	0.75 ± 0.05	0.44 ± 0.08	0.83 ± 0.03	0.24 ± 0.08	8.88 ± 0.01	3.01 ± 0.15	3.65 ± 0.04	1.25 ± 0.05
	T3	0.69 ± 0.01	0.51 ± 0.11	0.69 ± 0.14	0.23 ± 0.09	8.85 ± 0..05	2.87 ± 0.18	3.66 ± 0.06	1.21 ± 0.01
PBW 621	C	1.79 ± 0.06	1.65 ± 0.12	0.86 ± 0.05	0.54 ± 0.16	9.18 ± 0.05	3.78 ± 0.08	3.52 ± 0.03	1.11 ± 0.01
	T1	0.46 ± 0.04	0.29 ± 0.15	0.35 ± 0.09	0.19 ± 0.02	8.08 ± 0.06	3.12 ± 0.05	2.35 ± 0.01	0.98 ± 0.03
	T2	1.61 ± 0.01	1.42 ± 0.09	0.91 ± 0.04	0.62 ± 0.13	9.21 ± 0.04	3.01 ± 0.11	3.62 ± 0.06	0.67 ± 0.14
	T3	1.68 ± 0.04	1.46 ± 0.17	0.85 ± 0.09	0.55 ± 0.25	9.19 ± 0.11	3.21 ± 0.24	3.68 ± 0.12	0.75 ± 0.12
CD at 5%	A	0.09		0.12		0.12		0.08	
	B	0.12		0.17		0.17		0.11	
	C	0.09		0.12		0.12		NS	

Values are mean ± SD of three replications. Control (C) – 17 mM glutamine + 117 mM sucrose, T1 – 5 mM glutamine + 117 mM sucrose, T2 – 25 mM glutamine + 117 mM sucrose, T3 – 125 mM sucrose + 17 mM glutamine, A – genotypes, B – treatments, C – stages.

Table 2. Effect of glutamine and sucrose at different levels on sucrose synthase (SS, µmol sucrose min^{-1} mg^{-1} protein), soluble acid invertase (AI, µmol sucrose min^{-1} mg^{-1} protein) and soluble neutral invertase (NI, µmol sucrose min^{-1} mg^{-1} protein) activities in grains of two wheat genotypes (PBW 343, PBW 621) at 4 and 7 days after culturing

Genotypes \ Enzymes		SS		AI		NI	
		4	7	4	7	4	7
PBW 343	C	101.6 ± 0.11	90.56 ± 0.06	62.12 ± 0.08	52.48 ± 0.15	38.5 ± 0.09	89.63 ± 0.06
	T1	95.51 ± 0.14	75.25 ± 0.09	35.63 ± 0.06	18.62 ± 0.17	30.55 ± 0.04	60.17 ± 0.02
	T2	99.21 ± 0.02	81.54 ± 0..04	36.78 ± 0.02	20.13 ± 0.09	29.76 ± 0.05	21.01 ± 0.01
	T3	115.8 ± 0.04	104.3 ± 0.05	61.73 ± 0.12	41.16 ± 0.04	68.21 ± 0.01	43.74 ± 0.03
PBW 621	C	99.18 ± 0.02	95.63 ± 0.08	62.07 ± 0.01	56.86 ± 0.12	33.89 ± 0.03	76.73 ± 0.04
	T1	80.62 ± 0.13	60.49 ± 0.04	40.73 ± 0.17	20.96 ± 0.16	28.78 ± 0.05	51.59 ± 0.09
	T2	96.57 ± 0.11	89.14 ± 0.03	48.72 ± 0.08	33.65 ± 0.11	29.14 ± 0.03	30.65 ± 0.14
	T3	117.5 ± 0.08	104.2 ± 0.05	65.24 ± 0.04	49.83 ± 0.14	51.39 ± 0.05	38.01 ± 0.07
CD at 5%	A	0.08		0.12		0.07	
	B	0.11		0.17		0.10	
	C	0.08		0.12		0.68	

Values are mean ± SD of three replications. Control (C) – 17 mM glutamine + 117 mM sucrose, T1 – 5 mM glutamine + 117 mM sucrose, T2 – 25 mM glutamine + 117 mM sucrose, T3 – 125 mM sucrose + 17 mM glutamine, A – genotypes, B – treatments, C – stages.

Table 3. Effect of glutamine and sucrose at different levels on protein (mg/g DW), amino acid (mg/g DW) and nitrogen (%) content in grains of two wheat genotypes (PBW 343, PBW 621) at 4 and 7 days after culturing

Genotypes \ Enzymes		Protein		Amino acid		Nitrogen	
		4 days	7 days	4 days	7 days	4 days	7 days
PBW 343	C	12.56 ± 0.09	7.98 ± 0.13	0.84 ± 0.07	0.89 ± 0.08	5.96 ± 0.18	5.12 ± 0.14
	T1	12.23 ± 0.12	7.51 ± 0.11	0.51 ± 0.11	0.49 ± 0.03	5.44 ± 0.09	4.96 ± 0.16
	T2	13.12 ± 0.05	7.56 ± 0.09	0.79 ± 0.02	0.90 ± 0.07	6.11 ± 0.13	4.13 ± 0.04
	T3	12.31 ± O.15	6.56 ± 0.18	0.71 ± 0.01	0.61 ± 0.03	4.13 ± 0.06	2.76 ± 0.08
PBW 621	C	12.61 ± 0.11	7.59 ± 0.12	0.72 ± 0.08	0.81 ± 0.04	6.69 ± 0.14	5.96 ± 0.09
	T1	12.48 ± 0.08	6.63 ± 0.17	0.49 ± 0.14	0.51 ± 0.02	6.57 ± 0.12	5.48 ± 0.04
	T2	14.56 ± 0.16	7.99 ± 0.11	0.65 ± 0.03	0.55 ± 0.06	6.53 ± 0.17	4.56 ± 0.01
	T3	12.23 ± 0.09	6.89 ± 0.14	0.55 ± 0.04	0.42 ± 0.02	6.5 ± 0.12	4.23 ± 0.02
CD at 5%	A	0.13		NS		0.11	
	B	0.18		0.10		0.16	
	C	0.13		0.68		0.14	

Values are mean ± SD of three replications. Control (C) – 17 mM glutamine + 117 mM sucrose, T1 – 5 mM glutamine + 117 mM sucrose, T2 – 25 mM glutamine + 117 mM sucrose, T3 – 125 mM sucrose + 17 mM glutamine, A – genotypes, B – treatments, C – stages. NS stands for non-significant values among genotypes, treatments and stages.

Table 4. Effect of glutamine and sucrose at different levels on protein (mg/g DW), amino acid (mg/g DW), nitrogen (%), total sugar (mg/g) and starch (%) content in grains of two wheat genotypes (PBW 343, PBW 621) at 4 and 7 days after culturing

Genotypes \ Enzymes		Total sugars		Starch	
		4 days	7 days	4 days	7 days
PBW 343	C	16.3 ± 0.16	12.8 ± 0.06	41.56 ± 0.09	62.66 ± 0.04
	T1	18.4 ± 0.19	15.4 ± 0.02	41.99 ± 0.13	64.56 ± 0.03
	T2	8.14 ± 0.13	5.12 ± 0.03	42.94 ± 0.11	64.13 ± 0.06
	T3	20.48 ± 0.15	11.64 ± 0.08	43.16 ± 0.13	65.61 ± 0.02
PBW 621	C	11.6 ± 0.10	11.67 ± 0.07	42.35 ± 0.13	62.98 ± 0.07
	T1	13.7 ± 0.13	11.7 ± 0.01	42.95 ± 0.09	62.96 ± 0.05
	T2	9.41 ± 0.04	7.13 ± 0.02	42.25 ± 0.16	64.79 ± 0.02
	T3	20.13 ± 0.06	14.13 ± 0.01	42.11 ± 0.18	65.84 ± 0.04
CD at 5%	A	0.10		0.10	
	B	0.14		0.15	
	C	0.12		0.10	

Values are mean ± SD of three replications. Control (C) – 17 mM glutamine + 117 mM sucrose, T1 – 5 mM glutamine + 117 mM sucrose, T2 – 25 mM glutamine + 117 mM sucrose, T3 – 125 mM sucrose + 17 mM glutamine, A – genotypes, B – treatments, C – stages.

respectively ($p \leq 0.01$), but showed negative correlation with starch content $r = -0.05$, -0.42, -0.15, respectively ($p \leq 0.01$). Whereas total protein, amino acid, nitrogen content were negatively correlated with starch content $r = -0.70$, -0.42, -0.77, respectively ($p \leq 0.01$).

Discussion

Apical stem culture technique is a direct mode of assimilate entry from vegetative tissue to reproductive structure. Therefore, in order to gain insight on grain filling processes, cell sap is manipulated with respect to assimilate entry into the grain. Apparently, results of this kind of study will give new insight to breeders and biotechnologist for enhancing yield production. Our results demonstrated that higher glutamine dose in culture medium lead to significant increase in nitrogen assimilation and aminotransferases activities that resulted in increased protein content as also reported by Hawkesford (2014). The increase in protein content was in parallel with activities of GOT and GPT as also reported by Asthir and Bhatia (2014). It seems that through enhancement of the activity of transaminases, the wheat grains can accommodate high level of exogenously-supplied amino nitrogen and transform it to protein as long as an adequate level of sucrose is present in the grain. However, soluble protein content significantly decreased at 7 DAC, while the soluble amino acid content showed a significant increase. Lin and Kao (2001) also observed that protein content decreased and soluble amino acids increased in NH_4Cl treated rice leaves which lead us to suggest that hydrolysis of proteins might be responsible for the accumulation of amino acids in wheat grains. Apparently, there is a glutamine-mediated

Table 5. Correlation coefficients between carbon and nitrogen metabolism at different glutamine and sucrose treatment of two wheat genotypes

Parameters	NR	GOGAT	GOT	GPT	SS	AI	NI	Protein	Amino acid	Nitrogen	Total sugars
GOGAT	0.712**										
GOT	0.302	0.694**									
GPT	0.287	0.754**	0.955**								
SS	0.453	0.627**	0.565*	0.631**							
AI	0.595*	0.733**	0.569*	0.591*	0.803**						
NI	0.177	0.029	–0.207	–0.134	0.093	0.371					
Protein	0.161	0.643**	0.968**	0.933**	0.427	0.431	–0.259				
Amino acid	0.757**	0.708**	0.166	0.211	0.187	0.523*	0.291	0.143			
Nitrogen	0.362	0.521*	0.621*	0.552*	0.021	0.277	0.149	0.651**	0.478		
Total sugars	–0.011	0.029	0.399	0.384	0.497*	0.451*	0.494*	0.284	–0.274	0.195	
Starch	–0.154	–0.512	–0.651	–0.623	–0.053	–0.424	–0.155	–0.707	–0.428	–0.773	–0.211

*, ** Significantly different at 5% and 1% level, respectively

diversion of sucrose and starch towards protein synthesis as observed earlier in rice grains (Singh et al. 1978). Since conversion of glutamine to glutamate by GOGAT in seeds needs 2-oxoglutarate which is derived from sucrose catabolism, therefore the higher concentration of glutamine causes greater generation of this organic acid (Hodges 2002; Lancien et al. 2002). As a part of this investigation, increasing concentration of glutamine in the culture medium results in higher content of nitrogen in PBW 621 which corresponds to increased activities of nitrogen assimilatory enzymes. All these processes enhanced the rate of glutamine uptake at the level of NR and GOGAT activities (Balotf et al. 2012; Hawkesford 2014). Apparently, PBW 621 also predominated with respect to enzymes of ammonia assimilation and aminotransferases (GOT and GPT) in grains as compared to PBW 343 showing thereby, higher assimilation of N, which ultimately results in high protein content.

From our result, protein and starch were inversely correlated indicating a compensatory effect on carbon and nitrogen metabolism as also reported by Khan et al. (2008). Metabolism of sucrose via soluble acid invertase predominated at 4 DAC while neutral invertase was more active at 7 DAC. Sucrose synthase activity in the synthesis direction was decreased from 4 DAC to 7 DAC as compared to invertases indicating that two hexoses (glucose and fructose) generated from the sucrose molecule are released by the action predominantly via invertase and hence incorporated into amino acids and protein (Koch 2004). Increase in starch content was more pronounced in grain of PBW 621 as compared to PBW 343 which may be due to higher stimulation of grain sink activity leading to increased dry matter accumulation. Thus, the sucrose content and SS activity in grains alone with the increase in nitrogen assimilation are important for the synthesis of both starch and protein, depending on the balance between carbon and nitrogen substrate in grains (Ibrahim et al. 2011). Higher glutamine concentration causes a shift in the balance of carbon and nitrogen metabolism by decreasing sugars and starch content, indicating funneling the accumulating sugars into respiratory metabolism generating carbon skeletons for amino acid biosynthesis (Parween et al. 2011). Therefore, manipulation of sucrose and amino nitrogen in the sap entering the seed is one way of potentializing the regulation of seed composition in wheat with respect to protein and starch contents (Allen and Young 2013).

Nitrogen uptake and assimilation were severely affected by higher sucrose concentration. Interestingly, upregulation of aminotransferases with increasing sucrose concentration was also reported by Rosales et al. (2011). Though the sugar content continued to increase to greater extent in the grains of PBW 343 with increasing concentration of sucrose in culture medium, however the activities of sucrose metabolizing enzymes showed a differential response. For instance, higher activity of SS (synthesis direction) in PBW 343 resulted in higher buildup of sugars in grain due to its lower conversion to starch as compared to PBW 621. But the activities of acid and neutral invertases increased at higher concentration of sucrose indicating its superior mechanism in terms of carbon utilization. In PBW 343, conversion of sugars to starch was less due to low increase in activity of acid invertase over PBW 621. The sucrose stimulation of grain sink activity in this way may thus stimulate grain filling processes leading to increased sugar content (Ozturk et al. 2010). Although it has proven difficult to attribute different roles to invertase activities, it

is quite probable that the ability of wheat grain to express two types of invertases may permit them to utilize assimilates differently. While the increase in the activity of soluble neutral invertase could infact regulate the hexoses for grain growth during later stages of grain development whereas the acid invertase could control the assimilate influx at early stages by increasing the sucrose gradient (Koch 2004). This indicates that higher build-up of sugars in the transport stream is essential for operating the sucrose carrier at saturation kinetics and ensuring the mass effect of sucrose for its efficient unloading into the seed. Singh et al. (1990) reported that some sucrose is reconstituted from external solution arising out of the sucrose imported into the seed of wheat and cereals resulting in increased sugar and starch content. Therefore, higher sucrose concentration increases sucrose to starch conversion with the decrease in soluble protein content. The results, therefore suggest that in wheat, the seed composition with respect to starch and protein contents can be regulated through alterations in the concentrations of sucrose and amino nitrogen in the transport stream entering the seed.

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