Phytochemistry, Vol. 21, No. 6, pp. 1183–1187, 1982.
Printed in Great Britain.

0031-9422/82/061183-05$03.00/0

PHOTOSYNTHESIS AND TRANSLOCATION RATE IN HIGH LYSINE MUTANT BARLEY

I. M. SANTHA, S. L. MEHTA, K. R. KOUNDAL* and S. K. SINHA

Nuclear Research Laboratory and *Water Technology Centre, Indian Agricultural Research Institute, New Delhi 110012, India

(*Revised received* 11 *August* 1981)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; Notch-2; NP 113; B_1; photosynthesis; translocation; changes during development.

Abstract—Photosynthesis and translocation rates were studied in high lysine barley mutant Notch-2 and its parent NP 113. Photosynthesis rates were higher in the mutant than in the parent variety during early growth but were lower 24 days after anthesis. Higher photosynthetic rates were also observed in hiproly out-cross B_1 than NP 113 during early development. However, on a per leaf basis, the photosynthetic rate in B_1 flag leaf was lower than in NP 113. [^{14}C]Sucrose translocation rates from the leaf to the developing ear were lower in mutant Notch-2 than in NP 113 or hiproly B_1. A substantially higher proportion of label remained in the flag leaf of Notch-2 than in NP 113 and B_1 at maturity. The decrease in yield in mutant Notch-2 may be caused by a lower rate of photosynthesis as well as reduced translocation rates during the later stage of grain development.

INTRODUCTION

Barley mutant Notch-2 induced by ethyl methane sulphonate of NP 113 seeds has substantially improved quality[1, 2] but reduced grain yield due to a decrease in the starch[3, 4]. Recent studies of enzymes of starch metabolism have shown that the activity of starch synthetase (EC 2.4.1.21) and phosphoketoisomerase in Notch-2 mutant grains is lower than NP 113[5, 6]. Based on the absolute activity of enzymes of starch metabolism and kinetic studies, a regulatory control on starch synthesis in Notch-2 has been suggested [7]. It has been shown that in cereals, sucrose is the major sugar transported from leaf to the grain[8]. Photosynthetic and translocation rates may also limit the supply of soluble sugars for the synthesis of starch and other constituents. Therefore, in the present study, the photosynthetic rate and efficiency of utilization of exogenously supplied sucrose have been examined in mutant Notch-2, its parent NP 113 and hiproly B_1 during development.

RESULTS AND DISCUSSION

The results presented in Table 1 show the photosynthetic rate per g fr. wt and on per leaf basis in NP 113, Notch-2 and B_1 leaves during development. During early development the photosynthetic rate per g fr. wt was higher in B_1 and Notch-2 leaves than in NP 113. The photosynthetic rate, both on per g fr. wt and per leaf basis, was higher in the mutant than its parent on day 30 and at anthesis, while it was substantially lower 24 days after anthesis. A lower photosynthetic rate on a per flag leaf basis in B_1 than in NP 113 was mainly due to the smaller size of the leaf of B_1.

The fr. wt of Notch-2 leaves was slightly lower than the parent NP 113 during development (Table 2). The dry wt of the flag leaf of Notch-2 differed little from that of NP 113 except at 24 days after anthesis, when it was lower. The fr. wt and dry wt of B_1 leaves were lower than both NP 113 and Notch-2 except at 24 days after anthesis.

Dry wt accumulation

The dry wt accumulation in Notch-2 was less than in NP 113 and B_1 throughout grain development (Fig. 1). The dry wt accumulation rate per day per grain (Table 3) during development was also lower in the mutant than in its parent. The maximum rate of dry wt accumulation was observed from day 17 to day 24

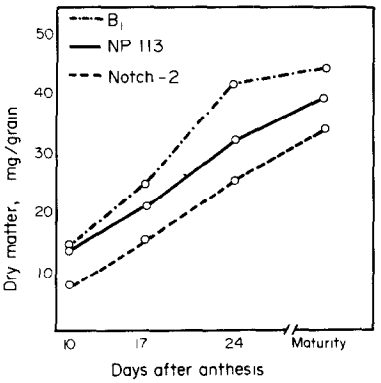

Fig. 1. Dry wt accumulation in grains of NP 113, mutant Notch-2 and B_1 barley during development.

Table 1. Photosynthesis rate in NP 113, Notch-2 and B_1 barley during development

Stage of development	mg CO_2 per g fr. wt per hr			mg CO_2 per leaf per hr		
	NP113	Notch-2	B_1	NP 113	Notch-2	B_1
1st leaf at 30 day stage	20.57(± 0.92)	34.05(± 1.10)	44.62(± 1.24)	11.58(± 0.52)	17.30(± 0.56)	8.48(± 0.23)
Flag leaf at anthesis	10.45(± 0.27)	21.30(± 0.46)	13.14(± 0.42)	4.68(± 0.12)	8.80(± 0.19)	1.71(± 0.06)
Flag leaf at 12 DAA*	41.80(± 1.40)	43.02(± 1.15)	47.70(± 1.50)	17.77(± 0.59)	14.84(± 0.39)	4.77(± 0.14)
Flag leaf at 24 DAA*	22.20(± 0.56)	11.17(± 0.48)	13.17(± 0.60)	4.15(± 0.26)	1.32(± 0.06)	1.00(± 0.04)

Values in parentheses indicate the standard error.
*DAA = Days after anthesis.

Table 2. Fresh and dry weights of NP 113, Notch-2 and B_1 barley leaf during development

Stage of development	NP 113		Notch-2		B_1	
	Fr. wt (mg)	Dry wt (mg)	Fr. wt (mg)	Dry wt (mg)	Fr. wt (mg)	Dry wt (mg)
30 day stage	563	110	508	98	190	38
Flag leaf at anthesis	448	94	413	95	130	72
Flag leaf at 12 DAA*	425	90	345	93	100	70
Flag leaf at 24 DAA	187	61	118	44	76	71

*DAA = Days after anthesis.

Table 3. Rate of dry weight accumulation in NP 113, Notch-2 and B_1 barley grains during development

Days after anthesis	Dry weight accumulation (mg per grain per day)		
	NP 113	Notch-2	B_1
Anthesis–10	1.33	0.80	1.34
11–17	1.08	1.05	1.63
18–24	1.64	1.46	2.42
25–maturity	0.48	0.34	0.17

in all varieties. Hiproly B_1 grains had higher dry wt accumulation rates than NP 113 and Notch-2 up to day 24.

[^{14}C]*Sucrose uptake and translocation*

In order to determine translocation rates [^{14}C]sucrose was uniformly applied to the flag leaf at anthesis and the distribution of the label in flag leaf, peduncle, husk, rachis and grain determined thereafter up to maturity. Results are shown in Tables 4 and 5. Peduncle, rachis, husk + awns + glume accounted for only a small fraction. At day 10, most of the label in mutant Notch-2 remained in the flag leaf and only a small proportion of the label was translocated to the grain. In NP 113 46% of the label appeared in the grain. At day 17 the proportion of label in the grain relative to the label in the flag leaf increased in all three plants. However, Notch-2 flag leaf still had considerably higher label than NP 113. At maturity the flag leaf of Notch-2 still had nearly 50% of the label, while NP 113 and B_1 showed only 13.2 and 13.9% label, respectively. The ratio of label in grain to the label in leaf increased both in NP 113 and B_1 during development, while in Notch-2 it increased only up to day 24. During grain development the proportion of label in the soluble fraction of grains of NP 113 decreased at day 17 but did not change much thereafter, while in Notch-2 it followed an erratic pattern. During development, the proportion of label in the insoluble fraction increased in NP 113 and B_1, while in Notch-2 grains it increased up to day 24 and decreased thereafter at maturity.

Table 4. Per cent distribution of label in different parts of NP 113, mutant Notch-2 and B_1 barley 10 and 17 days after anthesis

Plant part	10DAA*			17 DAA		
	NP 113	Notch-2	B_1	NP 113	Notch-2	B_1
Husk + awns + glume	0.13	0.04	0.10	2.35	1.21	0.78
Rachis	0.05	0.02	0.03	0.61	0.70	0.35
Peduncle	0.49	0.21	0.58	6.63	4.28	5.61
Flag leaf						
Soluble	41.60	81.58	59.98	26.04	36.21	40.04
Insoluble	12.12	8.17	11.54	13.61	13.68	9.63
Grains of spike						
Soluble	17.07	5.94	9.95	12.46	20.45	7.19
Insoluble	28.54	4.03	17.80	38.30	23.50	38.28
Total counts incorporated	17.5×10^5	25.6×10^5	11.2×10^5	17.2×10^5	17.4×10^5	10.6×10^5

*DAA = Days after anthesis.

Table 5. Per cent distribution of label in different parts of NP 113, mutant Notch-2 and B_1 barley 24 days after anthesis and at maturity

Plant	24 DAA*			Maturity		
	NP 113	Notch-2	B_1	NP 113	Notch-2	B_1
Husk + awns + glume	1.31	0.56	0.50	1.54	0.69	0.78
Rachis	0.48	0.15	0.14	0.46	0.32	0.29
Peduncle	5.37	1.16	1.30	3.63	0.86	1.48
Flag leaf						
Soluble	21.47	38.39	25.30	8.71	36.17	10.87
Insoluble	9.16	11.17	9.93	4.50	12.84	3.06
Grains of spike						
Soluble	12.57	7.35	9.53	14.10	16.04	15.02
Insoluble	49.64	41.25	53.30	67.06	33.07	68.49

*DAA = Days after anthesis.

Discussion

Notch-2, the high-lysine mutant of barley, like other high-lysine cereal grain mutants, has a lower grain yield mainly due to reduced grain weight at maturity compared with its parent NP 113[9]. Starch and protein are the two major components of grain yield. It has been shown earlier that the protein content per grain in Notch-2 and NP 113 is nearly the same and that the reduction in yield is due mainly to the slower rate of starch synthesis during grain development[4] in mutant Notch-2. Sen and Mehta[3] have ruled out the possibility of an enhanced degradation of synthesized starch in the Notch-2 mutant compared with NP 113. In recent studies of enzymes of starch metabolism[5, 6], it has been suggested that the lower levels of ADP glucose (UDP glucose) starch synthetase and phosphoketoisomerase are responsible for the reduced accumulation of starch in Notch-2 grains compared with NP 113. In high-lysine Opaque-2 maize also, the decreased level of starch synthetase has been shown to contribute to the reduced endosperm weight[10]. Sucrose is the main raw material for the synthesis of starch in developing cereal grains[8]. The pattern of changes in reducing sugars in maize[11] and barley[12] are consistent with a mechanism by which starch is synthesized from a precursor pool of soluble reducing sugars. Therefore, starch synthesis may also be limited by the supply of the soluble sugars as substrate molecules. The high-lysine Notch-2 barley and B_1 leaves had a higher photosynthetic efficiency than NP 113 per unit wt up to 12 days after anthesis. At 24 days after anthesis both had much lower photosynthetic efficiency than NP 113. Notch-2 had a substantially higher photosynthetic capacity (CO_2 assimilated per leaf) than NP 113 up to anthesis, but at subsequent stages it was lower. The decreased photosynthetic capacity in mutant Notch-2 at a time when rapid starch synthesis takes place in the grain may therefore play an important role in limiting the starch accumulation. A higher label content in leaves of Notch-2 compared with NP 113 is suggestive of poor mobilization of photosynthates in the mutant. The findings of this study and our earlier investigations indicate that the grain yield reduction in this high-lysine mutant is caused not only by the lower levels of enzymes of starch biosynthesis but also by the lower rates of photosynthesis and translocation during later stages of grain development.

EXPERIMENTAL

Seeds of barley variety NP 113, its mutant Notch-2 and a hiproly derivative B_1 were grown at the Institute Farm under normal agronomic practices.

Photosynthesis rate. $^{14}CO_2$ generated from 10 μCi $NaH^{14}CO_3$ (sp. act. 54 mCi/mmol) by adding 2 ml 1M HCl was fed in a closed system. Six intact leaves were inserted in the Plexiglass chamber for 5 min. Air was circulated in the chamber by a pump. Immediately after feeding, the leaves were plunged in 80% EtOH and extracted successively with 80% EtOH, 50% EtOH and H_2O[13]. All extracts were pooled, acidified with HCl and the volume made up. Counting was done using toluene-based scintillator with a scintillation spectrometer. Values reported are an average of triplicated estimates.

[^{14}C]*Sucrose feeding studies.* 30 μl (7.5 μCi) [^{14}C]sucrose (sp. act. 495 mCi/mmol) was uniformly applied on each flag leaf of the plant on the day of anthesis. The plants of each variety were then harvested at 10, 17 and 24 days after anthesis and at maturity. Flag leaf, peduncle and the different components of the ear were separated. The flag leaves were washed thoroughly. The flag leaves and other parts were completely oven-dried at 60°. Radioactivity in the washings was determined. At day 10 *ca* 3.52 μCi ^{14}C was still on the outside of the flag leaf of NP 113 and Notch-2 and 3.75 μCi ^{14}C in the case of B_1, but at day 17 and subsequent stages washing accounted for < 0.1 μCi ^{14}C.

Extraction and fractionation. The distribution of label in the soluble and insoluble fractions of flag leaf and grains was determined. The soluble fraction from 50 mg samples in duplicate was extracted with 80% boiling EtOH (5 ml each time $\times 4$). The residue after centrifugation at 8000 *g* for 10 min of the last extraction was further treated with 4M HCl and autoclaved at 1 atm for 10 min. The digested material was filtered and an aliquot used for counting the insoluble fraction. An aliquot of the pooled EtOH extract was used for counting the soluble fraction. Peduncle, husk + awns + glume and rachis were treated as for the insoluble

fraction of the grain. The counting was done in a scintillation spectrometer using dioxane-based scintillator. The results reported are an average of duplicate estimates.

Dry wt accumulation. Dry wt accumulation was determined as described in ref. [3].

REFERENCES

1. Bansal, H. C., Srivastava, K. N., Eggum, B. O. and Mehta, S. L. (1977) *J. Sci. Food Agric.* **28**, 157.
2. Balaravi, S., Bansal, H. C., Eggum, B. O. and Bhaskaran, S. (1976) *J. Sci. Food Agric.* **27**, 545.
3. Sen, K. and Mehta, S. L. (1980) *Phytochemistry* **19**, 1323.
4. Batra, V. I. P., Bansal, H. C. and Mehta, S. L. (1982) *J. Sci. Food Agric.* **33**, 30.
5. Batra, V. I. P. and Mehta, S. L. (1981) *Phytochemistry* **20**, 635.
6. Batra, V. I. P. and Mehta, S. L. (1981) *Phytochemistry* **20**, 1827.
7. Batra, V. I. P. (1980) Ph.D. Thesis, P. G. School, I.A.R.I.
8. Porter, H. K. (1962) *Annu. Rev. Plant Physiol.* **13**, 303.
9. Bansal, H. C. (1974) *Indian J. Genet.* **14**, 657.
10. Joshi, S., Lodha, M. L. and Mehta, S. L. (1980) *Phytochemistry* **19**, 2305.
11. Ozbun, J. L., Hawker, J. S., Greenberg, E., Lannel, C. and Preiss, J. (1973) *Plant Physiol.* **51**, 1.
12. Baxter, E. D. and Duffus, C. M. (1973) *Phytochemistry* **12**, 1923.
13. Shantha Kumari, P. and Sinha, S. K. (1972) *Photosynthetika* **6**, 189.