Original Research Article

Possibility of Using Leaf *in vivo* Nitrate Reductase Activity as a Biochemical Marker for Predicting Grain Protein Content of Pearl Millet

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A B S T R A C T

Keywords Pearl millet, Nitrate reductase, Grain protein, Biochemical marker	The investigation was carried out to find any relationship between *in vivo* leaf nitrate reductase (NR) activity at early vegetative stage and fully mature grain protein content. Seven designated B-lines (counter part of CMS lines), one composite and one advanced inbred line were analyzed for their *in vivo* leaf NR activity at panicle initiation stage (25 days before anthesis) and grain protein content. The leaf NR activity varied from 7.9 to 16.95 µg NO_2^- $g^{-1}h^{-1}$ during *kharif 2014* and protein content varied from 10.98 to 18.4% and 11.03 to 14.85% during *kharif-2013* and *kharif-2014* respectively. Very high significant positive correlation was observed in leaf NR activity at panicle initiation stage with grain protein content (r=0.700 to 0.756; P<0.01). This relationship of leaf NR activity and grain protein content in pearl millet is first report on the aspect.

Introduction

Nitrogen metabolism is not only one of the basic processes of plant physiology, but also one of the important parts of global chemical cycle. Plant nitrogen assimilation directly takes part in the synthesis and conversion of amino acid through the reduction of nitrate by nitrate reductase. This is a rate limiting enzyme in the series of reactions whereby nitrogen is utilized for protein synthesis by plants. Leaf NR activity is known to be influenced by available soil nitrogen, the amount of light, environmental factors and genetic composition of plant (Ingle *et al.,* 1966; Kannangara *et al.,* 1967; Duffield *et al.,* 1972), salinity (Albassam, 2001), nitrogenous fertilizers (Gupta *et al.,*

2012; Liyuan and Shi, 2013). While conducting the experiment, it was ensured that the plants were given equal treatment and were not under any kind of stress mentioned before. In some other cereal and pulse crops, it is well known that leaf NR activity at booting, heading or milking stages positively correlated with their grain yield and grain protein content (Hernandez *et.al.*, 1974; Yong-Jian *et al.,* 2009). But in pearl millet there are no such reports that could establish any correlation in grain protein content or grain yield with its leaf NR activity at early growth stage. The purpose of this investigation was to find relationship, if any, between leaf NR activity

at early growth stage and grain protein content in pearl millet in advance. This would help in screening of pearl millet germplasm for protein content.

Materials and Methods

Seven B-lines (counterpart of CMS lines) one composite and one advanced inbred lines of pearl millet were included in this investigation. These genotypes were grown in five rows each with 10cm intra-row and 45 cm inter row spacing at research farm of CCS HAU, Hisar during *kharif-2014*. The crop was shown on July 12, 2014 with 60kg/hectare nitrogen and 35 kg/hectare phosphorous application. At the time of sowing, complete dose of phosphorous and 14 kg nitrogen was applied, the remaining dose of nitrogen was applied in two split dose on 25 days after sowing and 45 days after sowing were applied after four days of respective irrigation. The samples were collected on August 25, 2014, i.e. four days after second irrigation but just before application of second dose nitrogen fertilizers from the field between 10.00 am to 11.00 am in full sunshine. Before proceeding for the sampling we standardized the position of leaf as well as the part of the leaf to be considered for *in vivo* leaf NR activity. For this experiment we selected fully opened 3rd leaf from the top and middle portion of the leaf after removing the mid rib for analysis. And from the same experiment grain samples were collected for protein estimation for *kharif-2014*. Protein content of the genotypes grown during *kharif-2013* was also taken in to consideration for working out the relationship.

The NR (E.C. 1.6.6.1.) *in-vivo* activity was determined by the method described by Sawhney and Naik (1972), for this, 250mg leaf discs were suspended in 10 ml of a medium constituting 50mM phosphate buffer (pH 7.5), 20 mM KNO_3 and 3% (v/v) n-propanol and then vacuum infiltrated till all the discs sank to the bottom of solution. These tubes were incubated in dark in an incubator at 30°C. After 0, 10 and 30 min, 1/10 of aliquot was withdrawn and the amount of NO^{-2} released was estimated by method described by Fewson and Nicholas (1961). The results were expressed as µg $NO^-_2 h^{-1} g^{-1}$ fresh weight. Total protein content was measured by micro-Kjeldahl's method (AOAC, 1990). Analysis of variance and correlation matrix was carried out with OP STATE software, available online with CCS HAU website (www.hau.ernet.in).

Results and Discussion

Before proceeding to the main experiment, selection of leaf position as well as part of the leaf to be considered for *in vivo* leaf NR activity was standardized. For this, five whole plants of each HC 20 and WHC 901-445 genotypes were randomly selected and *in vivo* leaf NR activity was determined simultaneously in 3rd, 4th, 5th and 6th leaf (starting top to bottom) of each plant. It was observed that *in vivo* NR activity was decreased with the age of the leaf i.e. 3rd leaf had highest, while 6th leaf had lowest NR activity irrespective of the genotype (Table 1). The NR activity varied from 8.67±0.27 to 2.81±0.20 µM NO_2^- $g^{-1}h^{-1}$ in HC 20 and from 10.45±0.15 to 3.64±0.20 µM NO_2^- $g^{-1}h^{-1}$ in WHC 901-445, respectively. Therefore, the 3rd leaf was selected for further study. For consideration of leaf portion, the selected 3rd leaf from both the genotypes was cut in two pieces width wise and *in vivo* NR activity was measured simultaneously in both the portions. The lower portion of the leaf showed higher enzyme activity than that of the top portion, irrespective of the genotypes. It was 11.35±0.27 µM NO_2^- $g^{-1}h^{-1}$ and 13.20±0.23 µM NO_2^- $g^{-1}h^{-1}$ in lower portion while 7.31±0.22 µM NO_2^- $g^{-1}h^{-1}$ and

9.55 ± 0.30 µM NO_2^- $g^{-1}h^{-1}$ in top portion of HC 20 and WHC 901-445, respectively (Table 2). Therefore, for further study, the middle portion of the leaf was selected for determining the enzyme activity.

The mean values of *in vivo* leaf NR activity and grain protein content is presented in table 3. Leaf NR activity of the tested genotypes varied from 7.9 µM $NO_2^-h^{-1}g^{-1}$ to 16.95 µM $NO_2^-h^{-1}g^{-1}$ during kharif 2014 and protein content varied from 10.98 to 18.4% and 11.03 to 14.85% during *kharif-2013* and *khari-2014,* respectively. Among all genotypes analyzed, HMS 18B exhibited the highest leaf NR activity during *kharif-2014.* Protein content of this genotype grown during *kharif-2013* and *khari-2014* was also higher among all the genotypes, whereas that of HMS 26Bwas lowest. Advance inbred WHC 901-445 had higher values for leaf NR activity as well as grain protein content than that of composite HC 20. Leaf NR activity at panicle initiation stage showed a significant positive correlation with grain protein content irrespective of the season on both scale, Spearman's Correlation Matrix (r=0.0.700-0.745; P<0.01) and Pearson's Correlation Matrix (r=0.731-0.756; P<0.01).

A positive correlation between protein content and NR activity of C_3 plants has been reported in literatue. Hernandez *et al.* (1974) reported a positive correlation between grain protein content with leaf NR activity at booting stage in wheat. The similar results were also observed in wheat by Dalling and Loyn (1977), Cory and Hageman (1970), Sharma *et al.* (2013) and in triticale by Moinuddin *et al.* (1996). Yong-Jian *et al.* (2009) had also reported similar results in rice with the maximum correlation at heading stage. Sawhney and Naik (1969) reported a wide variation in grain protein content, ranging from 7.87 to 20.12% on moisture-free basis, in different varieties/lines of pearl millet.

Table.1 *In vivo* NR activity in different leaves of two pearl millet genotypes

Leaf Position (Top to bottom)	NR ($\mu M NO_2^-$ $g^{-1}h^{-1}$)	
	HC 20	WHC 901-445
3rd	8.67±0.27	10.45±0.33
4 rd	6.57±0.32	8.55±0.30
5 rd	3.51±0.21	548±0.25
6 rd	2.81 ±0.20	3.64±0.15

Table.2 *In vivo* NR activity in different parts of 3rd leaves of two pearl millet genotypes

Leaf Part	NR ($\mu M NO_2^-$ $g^{-1}h^{-1}$)	
	HC 20	WHC 901-445
Lower portion	11.35±0.27	13.20±0.23
Upper Portion	7.31±0.22	9.55±0.30

Table.3 *In vivo* NR activity in 3rd leaf of pearl millet genotypes at 25 days before anthesis and grain protein content

| Pearl millet Genotype | NR activity ($\mu MNO_2^- g^{-1}h^{-1}$) Kharif 2014 | | Protein Content (%) | | | |
| | Mean | S.E. | Kharif 2013 | | Kharif 2014 | |
			Mean	S.E.	Mean	S.E.
HMS 7B-1	10.35	0.15	16	0.1	14.8	0.1
HMS 13B	10.75	0.28	14.25	0.05	14.125	0.075
HMS 18B	16.95	0.25	18.4	0.1	14.85	0.05
HMS 26B	7.9	0.28	10.975	0.125	11.35	0.1
HMS 53B	9.8	0.18	12.675	0.025	11.035	0.035
HMS 52B	8.9	0.13	12.95	0.05	11.33	0.07
HMS 14B	13.8	0.17	14.35	0.05	14.54	0.09
HC 20	11.09	0.12	12.725	0.075	11.92	0.08
WHC 901-445	13.49	0.25	13.195	0.095	14.54	0.19
C.D.	0.52		0.261		0.315	
SE(m)	0.16		0.081		0.097	
SE(d)	0.227		0.114		0.137	
C.V.	1.979		0.817		1.043	
Spearman's Rank Correlation (P<0.01)			0.700		0.745	
Pearson Correlation Matrix (P<0.01)			0.756		0.731	

Thus, *in vivo* leaf NR activity is genotypic specific. Duffield *et al.* (1972) in winter wheat and Gupta *et al.* (2012) in finger millet, have also reported genotypic variation in leaf NR activity. Therefore, the positive correlation between leaf NR activity and grain protein content gives an advantage to early selection of high grain protein containing pearl millet germplasm lines. NR activity might be used as biochemical marker for predicting grain protein content; however, a fair number of lines need to be tested to strengthen this conclusion. Extensive programme of characterizing pearl millet germplasm (approximately 1000 lines) for quality parameters is under way at CCS Haryana Agricultural University, Hisar. Once established measuring in vivo NR activity of the germplasm will be an advantage in terms of early selection of CMS lines and inbreds for protein content for developing hybrids with improved protein content.

Abbreviations

NR- Nitrate reductase, GS- Glutamine synthetase, GOGAT- Glutamate synthase

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