Changes in Nitrate Reductase Activity of Leaf and Nitrogen Distribution with Growth in Potato Plants

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Abstract : Four field-grown potato cultivars, Irish Cobbler, May Queen, Dejima and Nishiyutaka, were subjected to measurements of leaf nitrate reductase (NR) activity (*in vivo* assay) and the distribution of nitrogen during growth and development. Varietal differences in NR-activity averaged over a season were observed : Highest activity was Nishiyutaka ($6.08 \text{ NO}_2^- \mu\text{mol h}^{-1} \text{ g}^{-1}$ fresh weight) and lowest was May Queen ($4.92 \text{ NO}_2^- \mu\text{mol h}^{-1} \text{ g}^{-1}$ fresh weight). Slightly high NR-activities were observed during the 30 days of early growth in all cultivars, and as growth proceeded, they tended to fall. The NR-activity was positively correlated with the percentage total leaf nitrogen, and with nitrogen accumulated in the leaf. The NR-activity had no correlation with total dry matter or tuber dry matter. The accumulation of nitrogen in the leaves declined with age, whereas it increased in the tubers regardless of the cultivar. At the end of the season, all cultivars except Nishiyutaka accumulated almost the same level of nitrogen in the tubers. A significant linear correlation ($r=0.749^{***}$) was found between the natural logarithm of SPAD reading (relative chlorophyll content) and NR. Harvest indices for nitrogen showed that the partitioning of nitrogen to the tubers was higher in Irish Cobbler and May Queen than in Dejima and Nishiyutaka. There was no evidence to conclude that NR-activity has a direct effect on dry matter production in potato.

Key words : Chlorophyll reading, Nitrate reductase activity, Nitrogen accumulation, Nitrogen harvest index, Potato.

バレイショの生育に伴う葉の硝酸還元酵素と窒素分布の変化 : Hemal Dhammike FONSEKA・浅沼興一郎・一井眞比古 (香川大学農学部)

要 旨 : バレイショ 4 品種 (男爵薯, メークイン, デジマ, ニシユタカ) を供試し, 葉身の硝酸還元酵素 (NR) 活性を *in vivo* で測定し, また, 葉身と塊茎における窒素分布を生育の時期別に定量した. NR 活性に有意な品種間差異があり, 平均値で比較すると, ニシユタカで最高値 ($6.08 \text{ NO}_2^- \mu\text{mol h}^{-1} \text{ g}^{-1}$ 生体重), メークインで最低値 (同 4.92) を示した. すべての品種で萌芽後 30 日目までに高い活性を示し, 生育とともに低下する傾向がみられた. NR 活性は葉の全窒素含有率および葉の窒素集積量と有意な正の相関を示したが, 全乾物重や塊茎乾物重とは相関がみられなかった. 全品種とも生育に伴い葉の集積窒素が減少し, 一方で塊茎では増加し, 生育終期にはニシユタカを除く 3 品種でほぼ同程度の窒素が塊茎に集積した. また, 葉緑素計 (SPAD) 指示値の自然対数と NR 活性との間には有意な直線関係 ($r=0.749^{***}$) が認められた. 窒素量について計算した収穫指数は男爵薯及びメークインで高く, デジマとニシユタカで低かった. 以上より, NR 活性はバレイショの乾物生産と直接結びつかないものと結論した.

キーワード : 硝酸還元酵素, 窒素収穫指数, 窒素集積, バレイショ, 葉緑素計.

Yield response to nitrate, the major source of nitrogen for most of the crop plants growing in aerated soils, is well documented. This is dependent mostly on plant capacity and efficiency in N-assimilation, which includes uptake, reduction and transport¹⁰⁾. Nitrate is assimilated thorough a pathway involving nitrate uptake steps, and by two reductive steps catalyzed by the enzymes nitrate reductase (NR) and nitrite reductase (NiR). Of these two enzymes, NR is considered to be the rate-limiting step in NO_3^- assimilation because it initiates the reaction when NO_3^- is available³⁾. The current study focuses on the reduction and transport components of the

N-assimilation process.

Most annual plants rely on N-uptake during vegetative growth and through the nitrate reduction process, reduced N is stored in leaves mostly as ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) which accounts for ca. 50% of the protein in leaves^{1,8)}. Compared to nitrate N, reduced-N is often an important reserve material which is remobilized from vegetative parts and to the developing sinks⁸⁾. Beevers and Hageman²⁾ and Kapoor and Li⁷⁾ showed that the assimilation of NO_3^- N via NR in intact plants was mostly confined to the leaves. Since the synthesis of all enzymes in under genetic control,

specific enzyme activities could be used as one of the criteria for the selection of superior genotypes. Further, enzyme activity has been shown to vary within species^{7,15}. The level of NR-activity is heritable^{2,13} and correlated with total reduced-N as well as yield⁵. Wary and Abberton¹⁴ indicated in their review that there have been many attempts to relate NR-activity to yield. However, they offered a list of 13 fundamental aspects of nitrate assimilation and N-metabolism, those scientists understood very little or nothing. Therefore, the use of 'N-assimilation' as a yield indicator is still not known.

Since NR plays a key role in N-assimilation, the estimation of NR-activity in different genotypes, its change during the growth and nitrogen distribution in different plant parts during growth and development could be used to identify cultivars with high genetic potential and high efficiency in translating internal plant-N into tuber yield. For these purposes, changes in NR-activity and the distribution of N among different genotypes were examined.

Materials and methods

Tubers of four potato cultivars, Irish Cobbler, May Queen, Dejima and Nishiyutaka were cut into pieces (30~50 g) and planted in an experimental field at the Faculty of Agriculture, Kagawa University on March 15, 1995 at a spacing of 66 × 30 cm, fertilized with 10.1 g N, 21.2 g P and 9.8 g K m⁻². The split plot design (1.98 × 1.8 m subplots) with two replicates was adopted. Both May Queen and Irish Cobbler showed shorter stem lengths, rapid initial growth and early leaf senescence compared to Dejima and Nishiyutaka. Samplings were done to cover five stages during the growth and development. Fully expanded leaves (the third and fourth leaf from the apex) were combined (four replicates) and used for *in vivo* nitrate reductase assay described by Kapoor and Li⁶. At each sampling, four plants were uprooted and separated into different organs, dried at 60°C for three days and finely ground to determine total N using CHN Corder (Yanagimoto Co., Japan). Nitrogen accumulation was calculated by multiplying %N with dry matter produced (g plant⁻¹) divided by 100. The relative chlorophyll contents of the most distal leaflets of third and fourth leaves

from 16 plants per cultivar at five growth stages were measured using a portable chlorophyll detector (SPAD 502, Minolta Co. Ltd., Japan). The LSD for mean separation and pooled correlations (n=20) was calculated with MSTAT-C, Michigan State University, U.S.A.

Results

1. Nitrate reductase (NR) activity

The NR-activity of four potato cultivars during the growth and development is presented in Fig. 1. Peak NR-activity for Dejima and Nishiyutaka was recorded at 30 DAE, while the peak for Irish Cobbler and May Queen was at 20 DAE. Differences between the mean NR-activities of these two groups were significant (LSD_{0.05}=0.406). The highest mean activity over the growth stages was recorded by the cultivar Nishiyutaka (6.08 NO₂⁻ μmol h⁻¹ g⁻¹ fresh weight), while the lowest was by May Queen (4.92 NO₂⁻ μmol h⁻¹ g⁻¹ fresh weight). The decline in NR-activity in Irish Cobbler and May Queen occurred sooner than that in Dejima and Nishiyutaka. The NR-activity at 50 DAE of Irish Cobbler and May Queen was almost half that of 10 DAE. The analysis of variance indicated the effect of cultivars was significant at the 0.05 level.

Fig. 1. Changes in nitrate reductase activity of four potato cultivars during the growing season. Bars indicate $\pm$ SE.
 —Δ—: Irish Cobbler, —▲—: May Queen,
 —○—: Dejima, —●—: Nishiyutaka.

Neither total dry matter, nor tuber dry matter, had significant correlation with NR-activity (data not shown).

2. Nitrogen content

The levels of percentage of total nitrogen (%N) were higher in the leaves than those in the tubers (Tables 1 & 2). Dejima and Nishiyutaka had higher %N in both leaves and tubers. All four cultivars showed declining trend in %N in the tubers with age. But the decline of %N in the leaves was observed to start at 20 DAE. The decline observed in %N of the tubers was not sharp compared to the leaves. Changes in %N in the leaves paralleled NR-activity. The correlation of NR-activity with %N in the leaves was significant, ($r=0.807^{***}$), whereas that with %N in the tubers was not ($r=0.409$).

3. Nitrogen accumulation in the leaves and tubers

Accumulation of total N in leaves and tubers expressed in g (dry wt.) per plant are shown in Tables 1 and 2. As to N-accumulation in the leaves, Irish Cobbler and May Queen showed a sharp decline, especially at the end of the season, while Dejima and Nishiyutaka showed a gradual decline. In contrast to the leaves, all cultivars tended to increase N-accumulation in tubers. At the end

of the season, all cultivars except Nishiyutaka had accumulated more or less similar levels of N in the tubers (Table 2). The correlation between nitrogen accumulation in the leaves and NR was highly significant ($r=0.676^{***}$). Dry matter accumulation in the tubers during the growth is shown in Table 2. Nishiyutaka, followed by Dejima, outyielded the other cultivars at the end of the growing season.

4. Chlorophyll (SPAD) reading

The plots of NR-activity and the SPAD reading shown in Figs. 1 and 2, respectively, indicate a division of cultivars into two groups. The SPAD readings attained maximum values at 30 DAE for Dejima and Nishiyutaka, and at 20 DAE for Irish Cobbler and May Queen, and were closely coupled in peaks NR-activity. A positive, significant correlation ($r=0.749^{***}$) was found between the natural logarithm of the SPAD reading and NR-activity (Fig. 3). Also, the SPAD reading showed significant positive correlation with % total N in the leaves ($r=0.825^{***}$).

5. Nitrogen harvest index (N_{HI}) of the component parts

The proportions of total plant N in the leaves, stems, and the tubers i.e. N-harvest indices (N_{HI}) were given in Table 3. In all cultivars, N_{HI} of the leaves were remarkably

Table 1. Percentage total nitrogen and N-accumulation (g plant⁻¹) of leaves of four potato cultivars during growth and development.

Cultivar	% total N					N-accumulation				
	DAE*					DAE				
	10	20	30	40	50	10	20	30	40	50
Irish Cobbler	3.82	5.68	4.70	4.63	2.71	0.35	1.10	1.11	0.81	0.22
May Queen	3.76	4.31	3.62	3.23	2.95	0.33	0.81	0.82	0.71	0.51
Dejima	4.68	4.87	5.34	4.85	3.19	0.31	0.94	1.23	1.26	0.73
Nishiyutaka	4.86	4.82	5.42	4.78	3.98	0.22	0.79	1.33	1.20	0.79

* : Days after emergence.

Table 2. Percentage total nitrogen, N-accumulation (g plant⁻¹) in tubers and tuber dry weight (g plant⁻¹) of four potato cultivars during growth and development.

Cultivar	% total N					N-accumulation					Tuber dry weight				
	DAE*					DAE					DAE				
	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50
Irish Cobbler	1.75	1.69	1.48	1.26	1.15	0.09	0.17	0.52	1.02	1.62	5.3	10.1	34.8	80.8	132.3
May Queen	1.76	1.59	1.31	1.26	1.08	0.06	0.16	0.65	1.03	1.70	3.1	10.2	49.9	81.7	139.8
Dejima	0.0	1.83	1.66	1.38	1.18	0.0	0.09	0.37	1.35	1.77	0.0	4.9	22.2	102.3	149.9
Nishiyutaka	1.82	1.69	1.61	1.38	1.34	0.03	0.15	0.63	1.13	2.11	1.6	9.2	39.2	81.5	157.7

* : Days after emergence.

higher until 20 DAE and thereafter decreased at accelerating rate. During the corresponding period, N_{HI} was high in the stems. But, after 20 DAE, the flow of N seemed to be predominantly directed towards developing tubers. This may be the cause for the observed decline of N_{HI} in leaves and stems. The proportion of total N found in the tubers of Dejima was comparatively lower than those of other cultivars as a fair amount of N was partitioned to the stems.

Discussion

NR-activity reached a peak during 20-30 DAE, and declined subsequently with age. The flow of N from leaves to tubers appears to be one of the reasons for the decline of NR-activity with age because NR has often shown to be a substrate inducible enzyme²⁾.

The decline of NR-activity with age has been reported in many plants, including potato^{4,7,11,13)}. We found two types of cultivars with different NR-activity at all growth stages and the leaf and tuber N contents closely followed the NR-activity in the leaves. A higher level of NR-activity may be responsible for higher N-accumulation observed in the tubers of Nishiyutaka and Dejima. This supports the concept that genetic potential for supplying reduced-N is reflected by NR-activity in leaves^{2,5,13)}. Data revealed that as NR-activity declines, the supply of reduced-N to the plant organs also decreased.

N-accumulation in leaves declined with age due to the remobilization of N from source to developing sinks, which was associated with the leaf senescence^{3,8)}. Thus, as leaves age and senesce, their capacity for photosynthesis de-

Fig. 2. Changes in relative chlorophyll content (SPAD reading) of four potato cultivars during the growing season.

Bars indicate $\pm$ SE. Symbols are the same as in Fig. 1.

Fig. 3. Relationship between relative chlorophyll content (SPAD reading) and nitrate reductase activity of potato leaves.

Table 3. Proportion of % total plant N in the leaves, stems and tubers of four potato cultivars during growth and development.

Cultivar	Leaves					Stems					Tubers				
	DAE*					DAE					DAE				
	10	20	30	40	50	10	20	30	40	50	10	20	30	40	50
Irish Cobbler	71.8	75.1	58.9	40.8	11.6	9.3	13.3	13.6	7.7	3.8	18.9	11.6	27.5	51.5	84.6
May Queen	79.5	76.4	51.8	39.3	22.5	7.1	8.5	7.0	3.6	1.6	13.0	15.2	41.2	57.1	75.9
Dejima	87.1	73.2	66.1	43.2	26.9	12.9	19.8	14.3	10.3	8.3	0.0	7.0	19.6	46.4	64.8
Nishiyutaka	81.0	67.8	59.5	46.5	25.9	8.4	19.0	12.3	9.7	4.4	10.1	13.3	28.2	43.8	69.6

* : Days after emergence.

clinences with a corresponding negative effect on assimilate supply and yield. On the other hand, flower production (data not presented), which was considered to be the trigger of leaf senescence⁶⁾, coincided with decline of NR-activity, N-accumulation, and SPAD reading of leaves in the present study.

It has been shown that the leaf N-status is closely reflected in the SPAD reading for many crops^{9,12)} as most of the N in the leaf is associated with protein in the chloroplast. There was a highly significant correlation between leaf-N-content and NR-activity, and the correlation found between SPAD reading and NR-activity in the present study can therefore be related to the close association between SPAD reading and the leaf-N-status of the leaves.

The N_{HI} for different organs of plants showed that factors such as assimilate partition and transport efficiency may exert a considerable effect on the accumulation of N in a particular cultivar. Although higher NR-activity is a prerequisite for higher protein (reduced-N) production, the present study suggests that other factors also play an important role.

It is well known that N plays a key role in the establishment and maintenance of the photosynthetic capacity of many crops. If no other restrictive factors are present, an increase in reduced-N supply through NR would increase the growth, chlorophyll content and photosynthetic capacity of leaves³⁾. Thus once plants are established, sufficient N must be available to maintain the photosynthetic apparatus and its activity. In the present study, both Dejima and Nishiyutaka showed higher NR-activity, higher %N and, as a result, a higher SPAD reading. In contrast, May Queen and Irish Cobbler showed low NR-activity, low %N and low SPAD reading. The gradual decline of NR-activity of Dejima and Nishiyutaka suggests that the established photosynthetic capacity is maintained for a longer period compared to May Queen and Irish Cobbler. The indirect effect on the maintenance process of photosynthesis may be responsible for higher assimilate supply and yield as in Dejima and Nishiyutaka. This supports the concept¹⁴⁾ that the effect of NR-activity on dry matter production is indirect. Therefore, our observation of cultivars

with higher NR-activity and higher dry matter would not necessarily support any hypothesis that higher NR-activity is the one key factor for higher dry matter productivity.

Therefore, it concluded that varietal differences in NR-activity exist and the tuber-N content is closely associated with NR-activity in the leaves of potato. Although high NR activity is associated with high N-content, it alone is not sufficient for a higher yield of genotype. Factors such as growth habit, photosynthetic efficiency, radiation use efficiency, transport efficiency, assimilate partitioning etc., may have considerable effect on the accumulation of dry matter and protein in a given genotype of potato.

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