Increasing world population is putting heavy pressure on the available arable land. The world’s total demand for food is likely to nearly double by 2030 from its present level, and there is limited new land available for expansion of cultivation to achieve this production level. Therefore, more food has to be produced from the presently available arable land rather than expanding the area under cultivation. Increasing crop yield potentials is an urgent concern. So, the higher nutrient use efficiency in plants must be fully explored to increase food production to feed the growing human population, and this has to be achieved without accelerating environmental degradation from excessive fertilizer use. Yield performance of any crop depends upon genotype and their interactions with environmental conditions. From the past century, conventional breeding has been proven successful in constantly raising the crop yield. This was mainly achieved with little or no knowledge of the factors governing the genetic variability of nutrient use efficiency exploited by breeders. Much research has been conducted to identify or breed nutrient efficient genotypes within species to further understand the mechanisms of nutrient efficiency in plants. However, success in releasing nutrient efficient genotypes has been limited due to complex genetics of such agronomic trait. The genetics of plant responses to nutrients and plant interactions with environmental variables are not well understood. Thus, complexity of genes involved in nutrient use efficiency for macro and micronutrients have hampered progress in this area (Baligar et al., 2001).

Most of the traits for nutrient use efficiency are quantitative and governed by quantitative trait loci (QTLs) and show continuous variation in natural populations. The genetic identification of traits has been difficult because heredity of traits controlled by polygenes is complex. During the three decades, advances in DNA technologies have led to dramatic achievement in the field of crop genomics. For example, DNA marker development resulted in marker assisted selection (MAS), which is widely applied to rice genomics because MAS effectively identifies the relationship between phenotype and genotype. Genomics assisted breeding relies upon the QTL identification, fine mapping, cloning, and its functional characterization to elucidate the mechanisms

KEYWORDS: Gene expression, nitrogen use efficiency, potato, real time PCR

GENE EXPRESSION ANALYSIS: INDICATORS OF NITROGEN USE EFFICIENCY IN POTATO CULTIVARS

Jagesh K Tiwari¹, SP Trehan², Sundaresha S¹, Poonam¹, BP Singh¹, VK Dua¹ and Vinay Bhardwaj¹

1ICAR-Central Potato Research Institute, Shimla- 171 001, HP, India.
Email: jageshtiwari@gmail.com
2ICAR-Central Potato Research Station, Jalandhar - 144 003, Punjab, India.

¹ICAR-Central Potato Research Institute, Shimla- 171 001, HP, India.
Email: jageshtiwari@gmail.com
²ICAR-Central Potato Research Station, Jalandhar - 144 003, Punjab, India.

Potato J 41 (2): July - December, 2014 175
underlying the traits. Subsequently, integration of additional molecular technologies like real time PCR and microarray technologies will allow agriculture scientists thoroughly understand the functional mechanism of genes resulting in more efficient crop breeding regimes (Fageria et al., 2008).

Among the major nutrients, nitrogen (N) plays a critical role in plant growth, as it is required for the synthesis of amino acids, proteins and DNA. Farmers usually try to increase crop yield by applying nitrogen containing fertilizers to the soil. However, this not only increases the cost of production, but also pollutes the environment. Therefore, boosting the efficiency of N assimilation and utilization in higher plants will have significant economic and environmental benefits. Recently, researchers at CPRI have identified Kufri Gaurav followed by Kufri Pukhraj as the nutrient efficient potato cultivars. The N use efficiency of these cultivars differs widely under N stress and sufficient conditions (Trehan, 2009). Therefore in the present study, real time gene expression of nitrate reductase (NR), nitrite reductase (NIR), ammonium transporter (AMT) and asparagine synthetase (AS) genes was analyzed in leaf tissues of three potato cultivars namely Kufri Gaurav (most N efficient in terms of both N uptake and utilization), Kufri Pukhraj (N uptake is equal to Kufri Gaurav but less N utilization efficiency) and Kufri Jyoti (least N efficient).

A field experiment was conducted using three Indian potato cultivars, Kufri Pukhraj, Kufri Gaurav (nutrient efficient) and Kufri Jyoti (least nutrient efficient) in the summer season (May to September, 2012) at CPRI Shimla. These cultivars were chosen based on differences in N use efficiency characteristics from screening/evaluation of cultivars in the field conditions (for details of N efficiency among these varieties, see the past study by Trehan, 2009). These varieties were tested under two levels of nitrogen (N0: without nitrogen, and N1: with 150 kg N/ ha) and replicated twice in RBD (replicated block design). Recommended packages of practices were followed to raise the crops (Trehan, 2009). The initial available N (alkaline KMnO₄ extractable) content of the soil was 227 kg ha⁻¹. In each replication, leaf tissue samples were collected in liquid nitrogen (-196°C) at 60 days after planting when potato tuber growth and development was in middle phase of growth.

Plant total RNA was isolated from 100 mg leaves collected from frozen leaf sample following manufacturer’s instruction using the RNeasy Plant Mini Kit (QIAGEN). cDNA synthesis was performed using 2 μg total RNA which was reverse-transcribed using TaqMan Reverse Transcription Reagent (Applied Biosystems, Branchburg, New Jersey, USA) (ABI). Consequently, cDNA was used for the gene expression study by real time-PCR analysis, using Power SYBR Green PCR Master Mix (ABI). Primers used for RT-PCR analysis is listed in Table 1 (Li et al., 2010). Real-time PCR analysis was followed as described in Sundaresha et al. (2014) using Cytochrome oxidase (coxI) as an internal standard gene following thermal cycler profiles: i) 50°C for 2 min; ii) 95°C for 10 min; iii) 40 cycles of 95°C for 15 sec, 60°C for 1 min, 72°C for 30 sec.

Among the three cultivars, Kufri Gaurav had the highest AMT activity in leaves under N0 treatment (Fig. 1). Kufri Pukhraj did not show higher activity of AMT in leaves than Kufri Jyoti under N stress. Results showed that Kufri Gaurav and Kufri Pukhraj differed in AMT activity in leaves under N stress and Kufri Gaurav had higher AMT activity than Kufri Pukhraj. Results further showed that these gene expressions better explained the N efficiency of potato
Gene expression analysis for N use efficiency in potato

Table 1. Primers used in RT-PCR analysis for nitrogen deficiency in potato.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium transporter</td>
<td>AMT-F</td>
<td>GGCGGCTTTAGTTTTAATCGGTGT</td>
</tr>
<tr>
<td></td>
<td>AMT-R</td>
<td>TTCCCTCCTCCACCGATTAAC</td>
</tr>
<tr>
<td>Asparagine synthetase</td>
<td>AS-F</td>
<td>AGCAAGCTGGACCTTG</td>
</tr>
<tr>
<td></td>
<td>AS-R</td>
<td>GGCACGCCCTACCATTTTG</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>NR-F</td>
<td>GGCAGAGCAATACCCAGATAG</td>
</tr>
<tr>
<td></td>
<td>NR-R</td>
<td>TCATAGAGCGTCCACCAAGC</td>
</tr>
<tr>
<td>Nitrite reductase</td>
<td>NiR-F</td>
<td>GTTCAGAAGCATAATTCCACAG</td>
</tr>
<tr>
<td></td>
<td>NiR-R</td>
<td>AGTTTAGACCTGCTGTACCTCC</td>
</tr>
<tr>
<td>Cytochrome oxidase (coxI)</td>
<td>coxI-F</td>
<td>TCCAGCCACAAAGGAGAAAGGC</td>
</tr>
<tr>
<td></td>
<td>coxI-R</td>
<td>CCGTTCGACCCGACCCCTACAC</td>
</tr>
</tbody>
</table>

Results showed that the most N efficient cv. Kufri Gaurav (Trehan et al., 2010) had the highest activity of this enzyme than Kufri Pukhraj and Kufri Jyoti (Li et al., 2010). It indicated that Kufri Gaurav is better equipped for ammonium utilization in the plant cells than Kufri Pukhraj and Kufri Jyoti. Both Kufri Gaurav and Kufri Pukhraj had higher activity of AS in leaves than the least N efficient cv. Kufri Jyoti (Trehan et al., 2010).

Fig. 1. Ammonium transporter (AMT) activity in leaves of three potato cultivars without and with N application.

Potato J 41 (2): July - December, 2014 177
particularly under N stress (Fig. 2). Highest activity of asparagine synthetase in leaves was recorded in Kufri Pukhraj followed by Kufri Gaurav and Kufri Jyoti under N0 (N stress) treatment. This enzyme catalyzes asparagine, one major function of which is to transport and store nitrogen according to the plant’s need. Results showed that both N efficient cvs. Kufri Gaurav and Kufri Pukhraj had higher activity of this enzyme than Kufri Jyoti. It indicated that both Kufri Gaurav and Kufri Pukhraj were better equipped for transportation and storage of N in plants than the least N efficient Kufri Jyoti. Whereas, in this present study, gene expression pattern in leaves of NR and NIR genes showed little variation in N efficient and N inefficient cultivars. Our results on the AMT and AS genes for determination of nitrogen efficiency in potato cultivars were corroborated with previous work. The past studies have helped in the identification of putative candidate genes encoding enzymes involved under nitrogen stress in potato. Recently, Zebarth et al. (2011) studied differential gene expression as an indicator of nitrogen sufficiency in field-grown potato cultivar ‘Shepody’ and concluded that an ammonium transporter gene (AMT) is as good as or better than conventional chemical or optical measures of potato N sufficiency. Li et al. (2010) demonstrated the potential to use gene expression markers of these genes (NR, NIR, AS and AMT) for early detection of nitrate deficient potato plant in nutrient culture by RT-PCR approach. Nevertheless, in the past many studies have been conducted in crop plants worldwide on nitrogen metabolism and candidate genes were identified. For example, Cai et al. (2009) studied glutamine synthetase (GS), a key enzyme that catalyzes the critical incorporation of inorganic ammonium into glutamine, and to name a few

It can be concluded that gene expression of AMT and AS in leaf tissues, particularly under N stress, successfully explained the variation of N efficiency in three cultivars.

![Fig. 2. Asparagine synthetase (AS) activity in leaves of three potato cultivars without and with N application.](image-url)
Gene expression analysis for N use efficiency in potato

namely Kufri Gaurav, Kufri Pukhraj and Kufri Jyoti, which differed widely in N efficiency as supported by yield data (Trehan, 2009). The higher expressions of these genes viz., ammonium transporter and asparagine synthetase in leaves can be used as indicators to screen plants/varieties/hybrids for high metabolism, utilization, transport and storage of nitrogen. To our knowledge, this is the first ever report on gene expression analysis for nitrogen use efficiency in the Indian potato cultivars.

LITERATURE CITED


MS received: 17 August 2013; Accepted: 04 December 2014