99. The genetic potential of wild and cultivated strawberries for nitrogen uptake and reduction

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Strawberry genotypes have been selected for a long time based on their fruit characteristics and their yield. Cultivated strawberries need a lot of nitrogen to grow and give a reasonable yield, because they have shallow roots which could not uptake nitrogen from deeper areas of the soil. Little attention has been paid on the efficiency of strawberry for taking up nitrogen and its reduction as nitrogen has been inexpensive, easily applied and typically is not a limiting factor in cultivation. However, as fertilizer prices rise and environmental impacts of nitrogen use become apparent, it is of interest to identify germplasm that may offer the ability to thrive with less nitrogen input. Many wild strawberries can thrive in rocky soils having little or no nitrogen. This germplasm may serve as a useful source of genes or alleles related to efficient nitrogen uptake or assimilation. In this study experiments have been conducted to study the physiological and genetic potential of wild strawberries and their efficiency in nitrogen uptake and reduction in comparison with cultivated strawberries. The genes identified can be transferred by conventional or modern biotechnology methods to strawberry for improving the cultivated strawberries for reducing nitrogen usage in the field and reducing nitrogen pollution of the environment.

Introduction

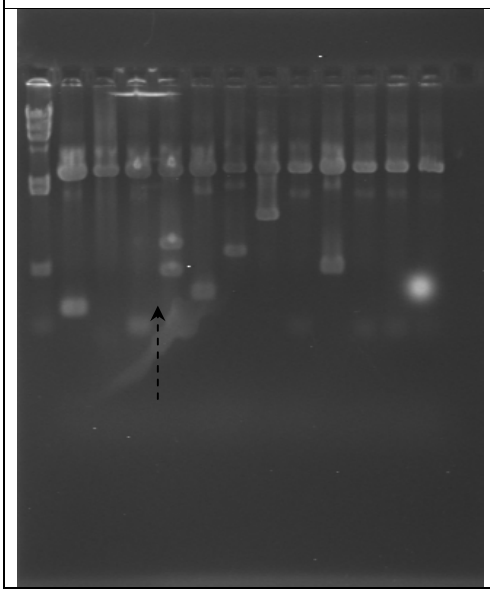
Nitrate act as a signal and a nutrient that supports and regulates plant growth, development, and metabolism (Crawford, 1995; Stitt, 1999; Crawford and Forde, 2002; Forde, 2002). It is a key enzyme that catalyzes the first step in plants nitrogen metabolization (Sun et al. 2008). Early studies on nitrate signaling demonstrated that nitrate induces de novo synthesis of nitrate reducatse (NR), the first enzyme in the nitrate assimilation pathway (Zielke and Filner, 1971; Somers et al., 1983; Remmler and Campbell, 1986). Subsequent work demonstrated that nitrate induces other genes in the nitrate assimilation pathway, namely, nitrate transporters (NRTs) and nitrite reductase (NiR), as well as genes involve in energy metabolism especially in the pentose phosphate pathway (Wang et al., 2000). When the NR gene is linked to cauliflower mosaic virus (CaMV) 35S promoter the NR gene is constitutively expressed, and NR mRNA is high (Vincentz and Caboche, 1991). Furthermore, it has been shown that over expression of NR gene can enhance the NR activity and reduce nitrate accumulation in *Nicotiana plumbaginifolia* leaves (Quillere et al., 1994). The same result has been seen in transgenic lettuce (Curtis et al., 1999).

In higher plants NR is a cytosolic enzyme encoded by a single nia gene. Till now, NR gene have been cloned from some plants such as *A. thaliana* (Cheng et al, 1988; Crawford et al., 1988), *Nicotiana benthamiana* (Vaucheret et al., 1989), barley (Miyazaki et al., 1991; Schnorr et al., 1991), maize (Hyde and Campbell, 1990), *Brassica napus* (Fukuoka et al., 1996) and *Ricinus communis* (Tsai et al., 2003). However, there were few reports about full-length sequence analysis and very little information available about NR gene in strawberry. In this paper, we have designed the primer based on the homology of NR gene sequence from *Arabidopsis* with *Prunus* or other family members from Rosaceae and try to compare the gene expression in wild type and cultivated strawberries to see the difference between them. The experiment is still under process.

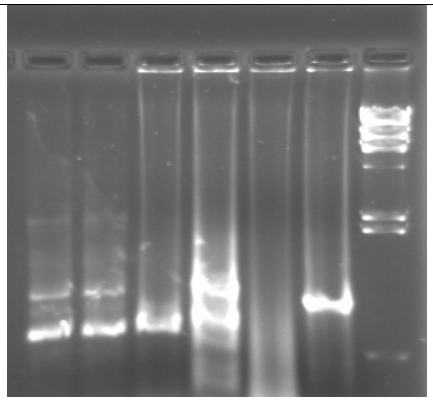
Material and Methods

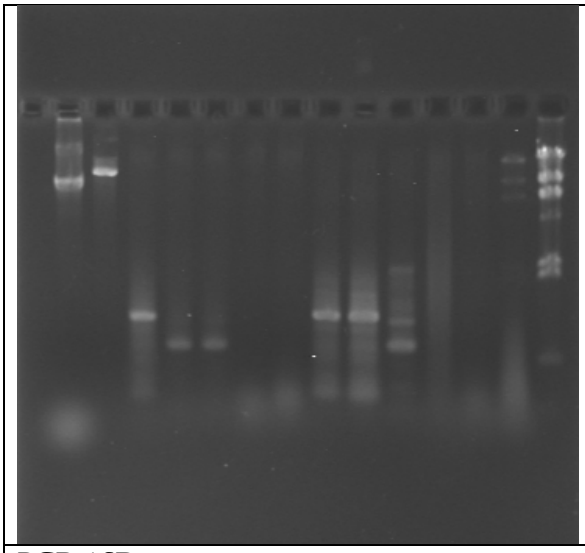
Different wild and cultivated strawberry plants were grown in greenhouse and allowed to produce runners. The runners then has been collected and rooted in soil or nutrient solution with 0.1 µM nitrate in nutrient solution. When the plants reached to a reasonable size they have dried with a paper towel and weighed. Then plants have been established in a nutrient solution containing MS micro and macro elements except nitrogen for at least 5 days to adopt the plants to new nutrient solution. At the start of the experiment root samples has been taken from the plants and then the plants were transferred to a new $1/4^{th}$ MS solution with 250 µM KNO_3. Another root samples has been taken at the 10, 30, 60, 120, 240 min intervals after the start of the experiment. The roots have been frozen in liquid nitrogen immediately after excision. Then RNA has been extracted from the roots by using CTAB and choloroform extraction method(?). The RNA then will be used for northern blotting and comparing the nitrate induced gene expression.

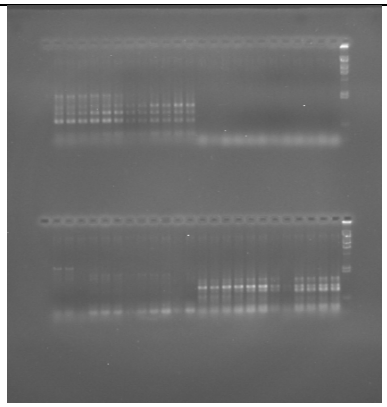
Sequence of *Arabidopsis* Nitrate Reductase (ANR1) gene has been compared with Rosaceae gene sequences and primers have been designed based on the homology between them. The polymerase chain reaction (PCR) has been done using the strawberry diploid genotype 5AF7 and the PCR product has been purified from the gel. The Product then have been transformed to a plasmid named pJET2.1/blunt, using CloneJET PCR Clonig Kit (Fermentas LIFE SCIENCES). The vector containing an expanded multiple cloning site, as weel as a T7 promoter for *in vitro* transcriptin. It expressed a ltheal restcirion enzyme after transformation and is not propagated. As a result, only the recombinant clones containing the insert appear on culture plates. Therefore, blue/white screening is not required.



PCR 15







PCR 16B

Results and Discussion

PCR amplification of strawberry genomic DNA has shown DNA sizes of 1000 bp.

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