# 1. Introduction

## 1.1 Nitrogen nutrition

Nitrogen (N) is a component of the fundamental building blocks of life (nucleotides, amino acids, and proteins) and therefore an essential nutrient for plant growth and development. Despite the great abundance of di-nitrogen gas ($N_2$) in the atmosphere, only few plant species are able to use it with the help of specific micro-organisms (Gordon et al. 2001). In the soil, N is available for the plants as inorganic N (e.g. nitrate and ammonium) and organic N (e.g. amino acids and urea) (Srivastava, Singh and Lea 1995, Jackson, Burger and Cavagnaro 2008, Crawford and Glass 1998). Tree growth depends upon adequate supply of N (Fisher and Garbett 1980, Binkley and Reid 1984). To stimulate plant growth millions of metric tons of N fertilizer is applied to the soil annually and the use of N fertilizer is expected to continually increase in the future because of the increasing demand of growing world population for food and fibre (Good, Shrawat and Muench 2004, Chardon et al. 2010).

N in the air and soil can be transformed into different chemical forms. For instance, nitrogen oxide (NO) emission can cause photochemical smog. NO can be oxidized to nitric acid, which leads to ecosystem acidification and eutrophication (Gruber and Galloway 2008). Excess N also has a negative influence on human health (Comly 1945, Gutiérrez 2012). Nitrate ($NO_3^-$) can be converted to nitrite ($NO_2^-$) by microbial action and further to nitrosamines, which have been considered as possible causative agents for human cancer (Lin 1990). All these possible detrimental effects make the understanding of plant N utilization an urgent issue to limit the use of N fertilizer (Xu, Fan and Miller 2012).

During the past decades, researchers have used genetic approaches to manipulate the expression of genes including the nitrate ($NO_3^-$) transporter genes (*NRT*) to improve N uptake. For example, over expression the nitrate transporter *OsNRT2.1* in rice suggested the role for this gene as an enhancer of vegetative growth (Katayama et al. 2009). Overexpression of another rice nitrate transporter (*OsNRT2.3b*) significantly increased yield and total N uptake (Xu et al. 2012). In the model plant, *Arabidopsis thaliana* overexpression of *NRT2.7*, a seed localized nitrate transporter, resulted in an increased $NO_3^-$ accumulation in seed and improved of germination rates (Chopin et al. 2007). Overexpression of *Arabidopsis NRT2.4* increased $NO_3^-$ content in plant biomass (Bertoni 2012). Furthermore, nitrate transporters are not the

only factors that influence N utilization. Examples include many other genes like ammonium transporters, nitrate assimilation related enzymes, transcriptional factors, etc. Xu et al. (2012) have summarized many impacts of these genetic factors on NUE (Table 1.1).

N fertilization has also been used of trees to improve biomass production and reduce rotation age of trees (Heilman and Norby 1998). However, it is notable that nitrogen utilization has not yet been fully addressed at a molecular point of view in poplar, the model plant for genetic analysis in tree species. The first step to address in this system is N acquisition. In this project, we focus on the relationship between the expression of nitrate transporter genes on the N status in poplar.

**Table 1.1 Transgenic approaches to improve plant N utilization** (Xu et al. 2012).

| Gene name (host plant) | Characteristics of N utilization (references) |
|---|---|
| **N transporters** | |
| *AtNRT1.1* (*Arabidopsis*) | $U_{Ni}$ ↑ (Liu, Huang and Tsay 1999) |
| *AtNRT2.4* (*Arabidopsis*) | $U_{Ni}$ ↑, shoot $NO_3^-$ ↑, DW ↑ (Bertoni 2012) |
| *NpNRT2.1* (Tobacco) | $U_{Ni}$ → in LN and HN, root $NO_3^-$ ↑ (Fraisier et al. 2000) |
| *OsNRT2.1* (Rice) | Shoot DW ↑, $U_N$ → (Katayama, Mori et al. 29) |
| *OsAMT1-1* (Rice) | Shoot and root DW ↓, $U_{Am}$ ↑ under LA and HA (Hoque et al. 2006, Kumar et al. 2006) |
| **Nitrate reductase, nitrite reductase** | |
| *NpNia2* (Potato) | TN ↓ 98 % (Dechorgnat et al. 2011, Djennane et al. 2002) |
| *LsNia* (Lettuce) | NR and $NO_3^-$ content ↑ in leaves (Curtis et al. 1999) |
| **Amino acid transporters** | |
| *ASN1/DglnAS1* (Tobacco) | Free asparagine in leaves ↑, growth rate ↑ (Brears et al. 1993) |
| *AtLHT1* (*Arabidopsis*) | Asp, Glu, and Gln uptake ↑; growth ↑ in LN (Herridge, Peoples and Boddey 2008) |
| *HvAlaAT* (*Arabidopsis*) | Seed yield ↑ 32.7 %, DW ↑ 55 % – 64 % in LN; DW ↑ 3 % – 75 % in LN (Good et al. 2007) |
| *AtASN1* (*Arabidopsis*) | Seeds TN ↑ in LN (Lam et al. 2003) |
| *AtAAP1* (*Arabidopsis*) | TN and C in seeds ↓, TAA ↑(Sanders et al. 2009) |
| **Glutamine synthetase** | |

| | |
|---|---|
| *PsGS1* (Tobacco) | Growth improved, leaves TAA ↓ (Oliveira et al. 2002) |
| *PsGS1* (Poplar) | Leaves DW ↑ (112 % in LN and 26 % in HN) (Man et al. 2005) |
| *PvGS1* (Wheat) | Root and grain DW ↑ (Habash et al. 2001) |
| *MsGS1* (Tobacco) | Shoot DW ↑ 7 % and root DW ↑ 100 % in LN (Fuentes et al. 200 |
| *OsGS1.1* (Rice) | Yield ↓ 25 % – 33 %; TN ↑ in both LN and HN (Cai et al. 2009) |
| *OsGS1.2* (Rice) | Yield ↑ 29 % – 35 % in HN; NUE ↑ 3 % – 33 % in HN (Brauer e 2011) |
| *ZmGS1* (Maize) | Shoot DW →, grain yield ↑ 45 % in LN; leaves TAA and TN ↑; g yield ↓ 85 % in LN (Martin et al. 2006) |
| *MsNADH-GOGAT* (Tobacco) | TC and TN in shoots ↑, DW ↑ (Habash et al. 2001) |
| *OsNADH-GOGAT* (Rice) | Grain filling ↑ (Kirk and Kronzucker 2005) |
| *MsNADH-GOGAT* (Alfalfa) | Shoot FM ↓ 29 % – 41 %, TN ↓ 37 % – 38 %, nodule TAA ↓ 5 % –7 % (Cordoba et al. 2003) |
| **Regulatory and transcription factors** | |
| *AtANR1* (*Arabidopsis*) | Insensitive to nitrate (Omari et al. 2010) |
| *ZmDof1* (*Arabidopsis*) | Growth rate ↑ in LN (Yanagisawa et al. 2004a) |
| **Others** | |
| *OsENOD93-1* (Rice) | Grain yield ↑ 10 % – 20 %, shoot DW ↑ 10 % – 20 %; TAA and TN in xylem sap ↑ in LN (Bi et al. 2009) |
| *APO1* (Rice) | Grain yield per plant ↑ 5 % –7 % (Terao et al. 2010) |
| *AtSTP13* (*Arabidopsis*) | TN ↑ 9 % and FW ↑ 75 % in HN (Schofield et al. 2009) |
| *AtPPDK* (*Arabidopsis*) | Rosette growth rate ↑ and seed weight and TN ↑ (Taylor et al. 2010) |

**Abbreviations:** AAP, aminoacidpermease; AlaAT, Alanineaminotransferase; AMT, ammoniumtransporter; ANR1, MADS transcription factor; APO, aberrant panicle organization; ASN, asparagine synthetase; Asp, aspartate; AspAT, aspartate amino transferase; At, *Arabidopsis thaliana*; CAT, cationic amino acid transporter; Dof1, dof transcription factor; DW, dryweight; ENOD, early nodulin; FW, fresh weight; Gln, glutamine; Glu, glutamate; GS, glutamine synthetase; HA, high ammonium concentration; HN, high nitrogen concentration; Hv, *Hordeum vulgare*; LA, lowammonium concentration; LHT, dysine histidine transporter; LN, low nitrogen concentration; Ls, *Lactucasativa*; Ms, *Medicagosativa*; NADH-GOGAT, NADH-dependent glutamate synthase; NAM-B1, NAC transcription factor; Nia, nitrate reductase (NADH); NiR, nitrite reductase; Np, *Nicotiana plumbaginifolia*; NR, nitrate reductase; NRT, nitrate transporter; Os, *Oryzasativa*; Pm, *Panicummiliaceum*; PPDK, pyruvateortho phosphate dikinase; RNAi, RNA interference; Pv,

*Phaseolusvulgaris*; STP, monosacchari detransporter; TAA, total amino acids; TC, total carbon content; TN, total nitrogen content; Ts, *Triticum dicoceoides*; Vf, *Viciafaba*; Zm, *Zeamays*; $U_{Am}$, ammonium uptake; $U_N$, nitrogen uptake; $U_{ni}$, nitrate uptake; ↑, increase; ↓, decrease; →, no change.

## 1.2 Nitrate transporters

### 1.2.1 Nitrate uptake system

The main inorganic N forms in the soil are nitrate ($NO_3^-$) and ammonium ($NH_4^+$) (Crawford and Glass 1998). The concentrations of $NO_3^-$ and $NH_4^+$ vary depending on the soil type and pH. In aerobic soil the major form of inorganic N is nitrate ($NO_3^-$); whereas in flooded wetland or acidic soil the major form is ammonium (Xu et al. 2012). Plant species differ in the use of a particular N source. It has been reported that many conifers prefer the utilization $NH_4^+$ (Min et al. 1998, Driessche 1971, Kronzucker, Siddiqi and Glass 1997). However, the majority of higher plants including poplar predominantly take up nitrate ($NO_3^-$) from soil as N source rather than ammonium ($NH_4^+$) (Crawford and Glass 1998, Rennenberg, Wildhagen and Ehlting 2010).

A large proportion of the $NO_3^-$ acquired by plants from soil is actively transported through $NO_3^-$ transporters (NRT) (Gojon et al. 2011). To cope with low (<1 mM) or high (>1 mM) $NO_3^-$ concentrations in soil plant roots have developed high-affinity (HATs) and low-affinity (LATs) nitrate uptake systems(Dechorgnat et al. 2010). Physiological studies further illustrate that each system is composed of constitutive and inducible components (Crawford and Glass 1998). Studies in *Arabidopsis* and other higher plants identified five gene families involved in nitrate uptake, allocation and storage: nitrate transporter 1/ peptide transporter (NRT1/PTR), nitrate transporter 2 (NRT2), nitrate assimilation related family (NRT3 or NAR2), chloride channels (CLC), and slow anion channel-associated 1 homolog 3 (SLAC1/SLAH) (Wang, Hsu and Tsay 2012b, Bouguyon, Gojon and Nacry 2012, Gojon et al. 2011, Tsay et al. 2007, Kotur et al. 2012). Generally, NRT1 family proteins are low-affinity nitrate transporters and NRT2 are high-affinity nitrate transporters. Two known exceptions are AtNRT1.1 of *Arabidopsis thaliana* and MtNRT1.3 of *Medicago truncatula*, which are dual affinity transporters (Criscuolo et al. 2012, Morère-Le Paven et al. 2011, Liu et al. 1999).

Following the uptake, the $NO_3^-$ assimilation takes place in roots and leaves, where it is reduced by the nitrate reductase to form nitrite, and further to ammonium. In herbaceous plants, nitrate is mainly reduced in the leaves because of the reductive power of photosynthesis (Pate 1980). The earlier researchers thought that the reduction of nitrate in woody plants mostly happens in the roots (Bray, 1983), but later high level of nitrate reductase activity were found in the leaves of some species, particularly among the gymnosperms, *Ericaceae* and *Proteaceae* (Smirnoff, Todd and Stewart 1984, Stewart, Hegarty and Specht 1988). This indicates that leaf is also an important organ for nitrate reduction in some woody plants. For example, a study on the partitioning of nitrate assimilation among leaves, stem, and roots in poplar revealed that little nitrate was assimilated in roots and the majority nitrate assimilation was taking place in leaves (Black, Fuchigami and Coleman 2002).

### 1.2.2 NRT1/PTR

There are 53 members in *Arabidopsis* belonging to the *NRT1/PTR* gene family. *NRT1.1* and *NRT1.2* have been shown to be involved in nitrate uptake (Tsay et al. 2007). The first nitrate transporter (CHL1 or NRT1.1) was identified in 1993 from a chlorate resistant mutant of *Arabidopsis* (Tsay et al., 1993). It encodes a protein with 12 trans-membrane segments (TMS) and takes part in both low affinity and high-affinity nitrate transport. The expression of *NRT1.1* can be induced by nitrate. The roles of NRT1.1 in root tips are not only being a nitrate transporter, but also a nitrate sensor to activate the expression of nitrate related genes in plants (Ho et al. 2009). Other members in NRT1 are low affinity nitrate transporters. *NRT1.2* is a constitutive low-affinity nitrate transporter expressed mainly in root hairs and epidermis (Huang et al. 1999).

Although the assimilation of nitrate can take place in roots, a large proportion of the assimilation happens in leaves (Wang et al. 2012b). It is known that nitrate assimilation is an energy-intensive process. The transportation of nitrate from roots to leaves enable the plants to directly use the energy and reductants derived from photosynthesis (Smirnoff and Stewart 1985, Smirnoff et al. 1984, Andrews 1986). In *Arabidopsis* three low affinity nitrate transporters (NRT1.5, NRT1.8 and NRT1.9) are involved in regulating root to shoot long-distance nitrate translocation (Wang et al. 2012b). NRT1.5 is a bidirectional nitrate transporter expressed in root pericycle cells close to xylem and participates in root xylem loading of nitrate (Lin et al. 2008). On the contrary, the NRT1.8 expressed predominantly in

the xylem parenchyma cells of *Arabidopsis* roots and removes nitrate from xylem vessels (Li et al. 2010a). AtNRT1.9 is expressed in the companion cells of root phloem and diminishes root to shoot nitrate transport (Wang and Tsay 2011b).

Some NRT1 proteins play a role in the nitrate distribution to leaves (Dechorgnat et al. 2010). The mutant *atnrt1.4* showed a reduction of nitrate content in petioles and mid-rips and an increase of nitrate content in the lamina (Chiu et al. 2004). It was suggested that AtNRT1.4 plays a crucial role in regulating leaf nitrate homeostasis. In addition, this study showed that AtNRT1.4 is not the only actor involved in this process. Two other NRT1 members (AtNRT1.5 and AtNRT1.8) have a function in nitrate loading and /or unloading to the shoot vasculature, too (Dechorgnat et al. 2010). Except those proteins, AtNRT1.7 is another one located in the plasma membrane of companion cells and the sieve element complex of mature leaves and have function to remobilize nitrate from older leaves to young leaves (Fan et al. 2009). Although there has been many progress in the characterization of the NRT1 genes, the function of woody member in this family is still unknown.

### 1.2.3 NRT2 and NRT3

Unlike the large family of NRT1, there are only seven NRT2 genes in *Arabidopsis*. Four of them (NRT2.1, NRT2.2, NRT2.4 and NRT2.7) have nitrate-related phenotypes in their mutants and can transport nitrate (Li et al. 2007, Chopin et al. 2007, Kiba et al. 2012). AtNRT2.1 is the major contributor among inducible HATs. AtNRT2.2 and AtNRT2.4 are the compensators of AtNRT2.1. The *Arabidopsis* AtNRT2.1 is expressed in epidermal, cortical and endodermal cells of mature roots (Nazoa et al. 2003). AtNRT2.2 only makes a small contribution to the plant nitrate transport. When AtNRT2.1 is lost by mutation, the expression of AtNRT2.2 will increase. This suggests a complementary function of AtNRT2.2 to AtNRT2.1. Double mutants (*Atnrt2.1-nrt2.2*) with the functions of NRT2.1 and NRT2.2 disrupted caused 80 % reduction of the inducible HATs and 30 % reduction of the constitutive HATs (Li et al. 2007). This indicates that multiple transporters may have similar or complementary functions in HATs. AtNRT2.4 is expressed in the epidermis of lateral roots and in or close to the shoot phloem (Kiba et al. 2012). It is also expressed in shoots mainly in primary veins of leaves (Bertoni 2012). The expression of NRT2.4 was undetectable in high N conditions and was activated under N starvation. (Bertoni 2012). The triple mutant of NRT2.1, NRT2.2, and NRT2.4 has a negative impact on biomass production under low nitrate

supply (Kiba et al. 2012). Most of AtNRT2 genes chiefly show expression in the roots, with the exception of AtNRT2.7, whose expression is higher in shoots than in roots (Wang et al. 2003). AtNRT2.7 plays a specific role in nitrate accumulation in the seed (Chopin et al. 2007).

Several recent papers document that the full function of NRT2 proteins is dependent on another family named NRT3. The NRT3, often referred to as nitrate assimilation related family (NAR2), which can form a two components complex together with NRT2 proteins to prompt high-affinity nitrate transportation (Plett et al. 2010, Li et al. 2010a, Yong, Kotur and Glass 2010, Kotur et al. 2012). The two component nitrate uptake system has been reported in many plant species, such as *Arabidopsis* (Yong et al. 2010), barley (Shinji et al. 2009), and rice (Feng et al. 2011, Kotur et al. 2012).

**Table 1.2 Summary of the physiological functions and expression of *NRT* genes in *Arabidopsis thaliana*.** ↑, induction; ↓, repression; →, constitutive; ?, not clear.

| Gene | Exp. tissues | Function | $NO^{3-}$ |
|---|---|---|---|
| *AtNRT1.1* | Root tips | High / low affinity $NO_3^-$ uptake; $NO_3^-$ sensing; high pH ↓; auxin, light, sugar, and nitrite (short-term) ↑ (Tsay et al. 1993, Guo, Wang and Crawford 2002, Wang, Xing and Crawford 2007, Lejay et al. 2008) | ↑ |
| *AtNRT1.4* | Leaf petioles and mid rid | Leaf $NO_3^-$ homeostasis (Chiu et al. 2004) | → |
| *AtNRT1.2* | Root tips; mature roots | Constitutive low affinity $NO_3^-$ uptake (Huang et al. 1999) | → |
| *AtNRT1.3* | ? | ?; light ↑ (Lejay et al. 2008, Okamoto, Vidmar and Glass 2003) | ↑ in shoot |
| *AtNRT1.5* | Roots; shoots xylem | Root xylem loading; high pH and low potassium ↓; Sugar ↑ (Lin et al. 2008); influencing the tolerance to salt, drought, and cadmium stresses | ↑ |
| *AtNRT1.7* | Leaves | $NO_3^-$ from old to young leaves; Sucrose ↑ (Fan et al. 2009) | ? |
| *AtNRT1.6* | Seed | Delivery of $NO_3^-$ to seed (Almagro, Lin and Tsay 2008) | ? |

| | | | |
|---|---|---|---|
| *AtNRT1.8* | Roots; shoots xylem | Xylem unloading; Cadmium ↑ (Li et al. 2010b) | ↑ |
| *AtNRT1.9* | Root phloem | $NO_3^-$ loading into root phloem (Wang and Tsay 2011b) | → |
| *AtNRT2.4* | Lateral roots; leaves (primary vein) | High affinity $NO_3^-$ uptake in root; $NO_3^-$ phloem loading; compensator of NRT2.1; ammonium ↓; light ↑ (Kiba et al. 2012, Lejay et al. 2008, Okamoto et al. 2003) | ↓ |
| *AtNRT2.1* | Mature roots | High affinity $NO_3^-$ uptake; $NO_3^-$ sensing; $NH_4^+$ and glutamine ↓; light and sugar ↑ ; regulate lateral root development (Nazoa et al. 2003, Zhuo et al. 1999, Munos et al. 2004, Li et al. 2007) | ↑ |
| *AtNRT2.2* | Roots | High affinity $NO_3^-$ uptake; compensator of NRT2.1 (Li et al. 2007, Cerezo et al. 2001, Okamoto et al. 2003) | ↑ |
| *AtNRT2.6* | Roots; shoots | Inoculation by phytopathogenic bacterium *Erwinia amylovora* ↑ (Dechorgnat et al. 2012) | → |
| *AtNRT2.5* | Roots; shoots | ? | ↓ |
| *AtNRT2.7* | Seed | $NO_3^-$ storage in seed (Chopin et al. 2007, Okamoto et al. 2003) | → |
| *AtNRT3.1* | Roots; shoots | Partner of NRT2 (Okamoto et al. 2006) | ↑ |

### 1.3 Regulatory functions of *NRT* family genes in plant tolerance of stress

High concentrations of N often increase the defenseless of plants to disease (Snoeijers et al. 2000). Some members of NRT1 and NRT2 showed various regulations by biotic stress. The *nrt2.1* null mutant was found to be less sensitive to a virulent strain of *P. syringae* in tomato (Camañes et al. 2012). This suggests that NRT2.1 in tomato may play a role in the resistance against pathogens. The expression of *NRT2.6* did not regulate by either N starvation or N supply in roots and shoots (Okamoto et al. 2003). However, its expression was induced after the inoculation of *A. thaliana* by the phytopathogenic bacterium *Erwinia amylovora*, and the decrease of *NRT2.6* transcript caused a lower tolerance to pathogen attack.

Nitrate reallocation to roots might be a common mechanism in regulating a wide range of stresses (Chen et al. 2012, Li et al. 2010a, Gojon and Gaymard 2010). Many studies showed that the nitrate reallocation process was regulated by NRT1.5 and NRT1.8, and the expression of *NRT1.5* and *NRT1.8* was oppositely regulated by a wide range of stresses. For instance, Li et al. (2010) found that NRT1.8-regulated nitrate distribution played an important role in $Cd^{2+}$ tolerance. The increase of nitrate in the roots of *atnrt1.5* mutants enhanced drought and salt tolerance (Chen et al. 2012). This study showed that the nitrate reallocation in *nrt1.5* extensively altered the expression levels of many marker genes in drought (*P5CS1*), salt (*HKT1 and SOS1*), and $Cd_2^+$ (*AtPCS1*) responsive pathways.

## 1.4 Nitrogen perception and signaling

N is not only an essential macronutrient, but also acts as a signal, which regulates plant gene expression, metabolism, and physiology. It influences plant growth and development (Gojon et al. 2011, Vidal and Gutierrez 2008, Gutiérrez 2012). Plants have developed complex regulatory mechanisms to deal with different N supply in soil. The regulatory mechanisms include local signaling pathways acting at the cellular level and also systemic signaling pathways communicating internal nutrient status across whole plant (Alvarez, Vidal and Gutiérrez 2012).

So far, the best-known nitrate sensor in *Arabidopsis* is NRT1.1 (CHL1). The study of an uptake and sensing decoupled mutant showed that the dual-affinity binding and phosphorylation switching of NRT1.1 enables the plant to sense a wide range of nitrate concentrations in the soil, thereby functioning as a nitrate sensor (Ho et al. 2009). Time series experiments revealed that *NRT1.1* was induced 20 minutes after 1 mM nitrate application.

AtNRT2.1 is another nitrate sensor in *Arabidopsis*. The expression of *AtNRT2.1* is induced by local nitrate supply and repressed by systemic feedback signals by high N status (Zhuo et al. 1999). Mutant studies also indicated that the phosphorylation and de-phosphorylation of NRT1.1 resulted in the regulation of NRT2.1 (Ho et al. 2009).

A hypothetical model of local and systemic N responses in *Arabidopsis* roots has been proposed in a recent review (Alvarez et al. 2012). This model states that the N nutrients and metabolites are perceived locally in different cell types. Upon N perception, local and

systemic N responsive signaling pathways are activated. Local signaling is associated with sensing N availability in the soil, mainly by NRT1.1. The expression of NRT1.1 in root tips and in internal layers of the root coincides with pericycle, stele and lateral root cap being the earliest and most responsive step. On the other hand, systemic signaling pathways informing the status of the whole plant are long-distance signals that move between different organs (Alvarez et al. 2012).

Auxin and cytokinin (CK) signaling pathways are vital players in N-regulatory networks. Auxin responds to nitrogen signals from shoot to root and enhances lateral root initiation and development (Fukaki and Tasaka 2009). In *Arabidopsis* quick changes of nitrate from high to low concentration increased auxin content in roots and stimulated lateral root growth (Walch-Liu et al. 2006). NRT1.1 and NRT2.1, two main nitrate sensors, are known to be hormone-responsive (Krouk et al. 2011). NRT1.1, the dual affinity nitrate transporter and sensor, is expressed preferentially in nascent organs and growing regions of roots and shoots in *Arabidopsis*, which suggests that NRT1.1 might be regulated by a growth signal such as auxin (Guo et al. 2002). NRT1.1 can facilitate cell-to-cell auxin transport (Krouk et al. 2010b). Low N availability might simulate the transport of auxin out of roots through NRT1.1 thereby repressing the *Arabidopsis* lateral root growth (Krouk et al. 2010c, Dechorgnat et al. 2010). Furthermore, auxin can also act as an important signal regulating NRT1.1 expression in roots and shoots (Guo et al. 2002).

Several lines of evidence suggest that CKs are necessary to integrate the N status of the plant and work as a systemic signal for communication between roots and shoots (Alvarez et al. 2012). CK content in the xylem of roots and shoots is induced by $NO_3^-$ supply, and thereby, regulates shoot growth (Gessler, Kopriva and Rennenberg 2004). On the other side, CK regulates the expression of N uptake related genes. NRT2.1 is supposed to be a key regulator of root architecture and its transcript level is strongly suppressed by both CK and auxin (Brenner et al. 2005, Krouk et al. 2011). Not only NRT2.1, but also NRT2.3 and NRT2.6 were found to be strongly regulated by CK (Brenner et al. 2005). The close correlation between nitrogen and CK in poplar suggests a potential role of CK in long-distance nitrogen signaling in poplar (Cline, Thangavelu and Dong-Il 2006).

## 1.5 Poplar as a model tree

The genus *Populus* emerged about 40 - 65 million years ago (Plomion et al. 2006) and is widely distributed over the northern hemisphere (Rennenberg et al. 2010). It consists of