

Why is phloem sap nitrate kept low?

1 | INTRODUCTION

It has now been 61 years since phloem sap composition has first been described, using sap extracted from willow (Peel & Weatherley, 1959). In that study, using colorimetric methods, nitrate was found to be undetectable. Several subsequent studies also reported the absence of nitrate in other species such as castor bean (*Ricinus communis*) (Hall & Baker, 1972) and overviews of phloem sap composition established the generally very low, or undetectable, concentration in phloem sap nitrate (Ziegler, 1975). Furthermore, experiments with ^{15}N -nitrate labelling in legumes have shown that nitrate could not be transported from xylem to phloem at detectable levels (Pate, Sharkey, & Lewis, 1975). There are more recent reports where nitrate was found to be undetectable, for example in maize (Lohaus et al., 2000). It is thus widely accepted that phloem sap nitrate is of negligible importance and in particular, that nitrate circulation from shoots to roots does not occur. For example, in a recent review, the backflow of nitrate via the phloem to regulate root development is not mentioned (figure 3 in Tegeder & Masclaux-Daubresse, 2018). Furthermore, in textbooks and university lectures, it is often reported that nitrate is absent from phloem sap and thus does not flow back from shoots to roots, see for example (Taiz, Zeiger, Moller, & Murphy, 2015). However, many pieces of recent evidence suggest the contrary and provide possible reasons explaining why phloem nitrate concentration is usually low. They are presented in this Opinion.

2 | PHLOEM SAP MAY CONTAIN NITRATE

In fact, there are noticeable exceptions where nitrate is not absent from phloem sap composition (Figure 1a–f). It is the case of cereals (wheat, rice), with phloem sap nitrate of up to 8 mM (Hayashi & Chino, 1985, 1986). In addition, in palm trees, nitrate has been found in phloem exudates for either trunk and inflorescence peduncles (van Die & Tammes, 1975). Extensive analysis of castor bean phloem sap has shown that the average nitrate phloem concentration is 0.59 mM, ranging from no detectability to 2.4 mM (Peuke, 2010). This is of course much less than in xylem, which contains up to 25 mM nitrate. Interestingly, nitrate is much more abundant in phloem (0.29 mM) than xylem sap (3 μM) in Western candle tree (*Banksia prionotes*) (Jeschke & Pate, 1995). These findings cannot be explained by the technique of sap collection (i.e., potential adulteration by other tissues upon sampling). The above-cited studies used very different techniques (aphid-stylet and pure phloem sap exudation) and therefore a

systematic contamination is unlikely. In effect, nitrate can be found in phloem sap using the aphid stylet method (i.e., involving stylectomy), which gives access to very high purity phloem sap (van Helden, Tjallingh, & van Beek, 1994).

3 | NITRATE REDISTRIBUTION VIA PHLOEM SAP CIRCULATION

Nitrate redistribution via phloem circulation has been recently suggested to occur in three physiological contexts (Figure 1g), supported by the characterization of nitrate transporters in *Arabidopsis*: remobilization from leaves, development of reproductive structures and nitrate backflow from shoots to roots.

3.1 | Nitrate transfer and remobilization in leaves

Three nitrate transporters are involved in nitrate transfer and/or redistribution to the phloem from xylem or source nitrate-containing tissues: NPF2.13/NRT1.7, NPF1.1/NRT1.12, and NRT1.2/NRT1.11 (Iqbal et al., 2020). In particular, it has been recently shown that NPF2.13/NRT1.7 is essential for remobilization of nitrate from old leaves to deliver nitrate into the phloem, making it available for new, developing leaves and enhancing nitrogen use efficiency (Chen, Chen, Tseng, & Tsay, 2020). NPF1.1/NRT1.12 and NPF1.2/NRT1.11 are involved in the transfer of xylem nitrate to phloem in the petiole thereby facilitating nitrate remobilization from mature (old) leaves to young leaves (Hsu & Tsay, 2013).

3.2 | Development of reproductive structures

Up to now, two nitrate transporters have been shown to be of importance in silique and seeds: NPF2.12/NRT1.6, and NPF5.5. NPF2.12/NRT1.6 is capable of transporting both nitrate and gibberellin $\text{GA}_{1/3}$, and is expressed intensively in funiculus vascular bundles, which are mostly made of phloem tissue in *Arabidopsis* (Almagro, Lin, & Tsay, 2008). In *NPF2.12/NRT1.6* mutants, mature seeds contain effectively less nitrate, suggesting that NPF2.12/NRT1.6 is involved in transporting nitrate from maternal tissue to the developing embryo via the phloem (Almagro et al., 2008). NPF5.5, which has been shown to transport nitrate, is involved in controlling embryo N content: in knock-out mutants, embryos at the bent cotyledon stage have a