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Assimilation and Transport of Nitrogen in Tulip (Tulipa gesneriana) as Pursued by ^{15}N

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Abstract
Characteristics of assimilation and transport of nitrogen (N) in tulip plants were investigated mainly by a ^{15}N tracer technique. Tulip roots accumulate a large amount of N (ca. 90 mgN/gDW) during winter, if N is available in the medium. The accumulated N in the roots originated mainly in absorbed N from the medium rather than from the bulb storage N. During the winter season, the accumulated N in some roots was not readily translocated to the other roots, suggesting that the nitrogen be accumulated independently in each root. The accumulated N remained in soluble forms, especially glutamine was a major constituent. After sprouting in spring, the accumulated N in the roots was rapidly consumed for growth of leaves and stems. Some part of this N was redistributed to bulblets as the plants got matured. The behavior of N accumulated in the roots during winter was basically similar to that of the bulb storage N. Thus, it may be concluded that the physiological role of the N accumulation in roots in to provide a sufficient amount of N required for a rapid growth of leaves immediately after sprouting. During this period, 4-methyleneglutamine was a major amino compound in leaves, stems and roots. The presence of this amide appeared to be a temporary storage of N in vegetative organs of tulip plants. The transport from of N in xylem was mostly glutamine, and 4-methyleneglutamine was not detected. A high accumulation (20-120 mg/gDW) of a novel organic acid, 2-ox-4-methyl-3-pentene-1, 5-dioic acid was found in leaves and stems. This acid seems to be a deamidation and deamination product of 4-methylenglutamine.

Discipline: Soils, fertilizers and plant nutrition/Horticulture
Additional key words: glutamine, labeled N, 4-methyleneglutamine, nitrogen metabolism, root

Introduction

Tulip is one of the most popular ornamental bulbous plants. However, the information on its plant physiology and metabolism has so far been rather limited[3]. In recent years, investigations on assimilation and transport of N in tulip plants have been made with a ^{15}N tracer technique. The present paper attempts to review results of those recent studies which have employed this technique.

The annual growth and senescence changes of tulip plants are shown in Fig. 1. After tulip bulbs are planted in autumn (A), fibrous adventitious roots grow rapidly from a basal plate of bulb (B). During winter, starch which is a principal reserve carbohydrate in bulb scales, is decomposed and the accumulation of sucrose and fractosylsucrose takes place[3,6,10,14]. In addition, a large amount of N is accumulated in roots during the winter season[1,2,7]. After sprouting in spring (C), leaves and a stem (flower stalk) grow rapidly (D). Flowering season is April or early May (E). After topping of a flower, new bulblets (daughter bulbs) grow fast and the bulblets can be harvested in June (F).

Abundance of ^{15}N in sampled plants was determined with emission spectrometry[5].

Nitrogen accumulation in roots during the winter season

Baba and Ikarashi observed an extraordinary high accumulation of N (maximum 8–9% dry weight bases) in tulip roots during the winter season[1,2]. The amount of N in roots accounted for over 50% of the total N in tulip plants during winter. Thus, tulip roots appear to be a major N storage organ before sprouting.

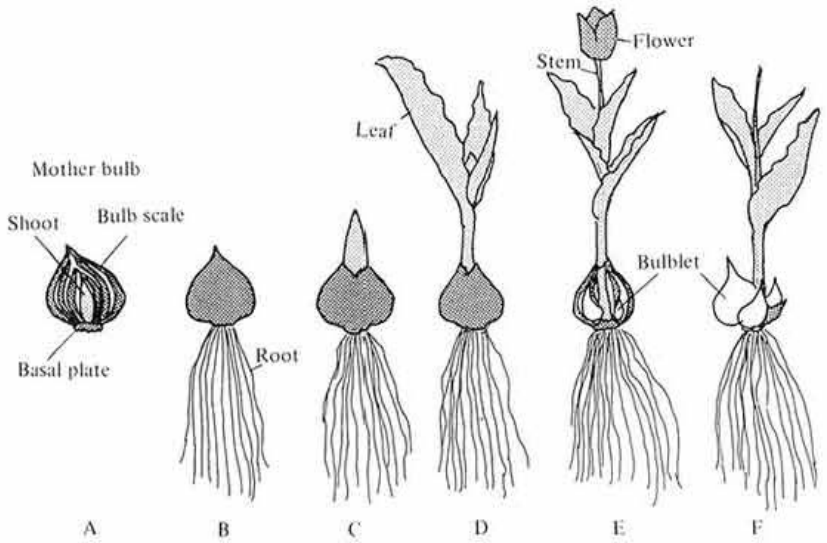

Fig. 1. Annual growth and senescence changes of tulip plants cultivated in Japan
A: Planting (Oct.), B: Root elongation and N accumulation (winter), C: Sprouting (Mar.), D: Leaf expansion (Mar.–Apr.), E: Flowering (Apr.–May), F: Harvesting (Jun.).
Source: Unpublished.

Table 1. Distribution of nitrogen in each fraction in the roots of tulip plants cultivated in the +N and –N medium

Fraction	N content (mgN/gDW)			Distribution of N (%)	
	+N (A)	–N (B)	A–B	+N	–N
80% ethanol soluble fraction	42.8	6.5	36.3	51	30
Water soluble but ethanol ionsoluble fraction	24.6	1.3	23.3	29	6
Insoluble fraction	17.2	14.2	3.0	20	64
Total amount of N	84.6	22.0	62.6	100	100

Source: Ohyama et al. (1985)[7].

Using a solution culture technique, tulip plants were grown with and without N (+N and –N treatments)[7]. As shown in Table 1, high accumulation of N (85 mg/gDW) in roots was found only when the plants were cultivated with +N medium (NH_4^+–N: 3 mg/*l*, NO_3^-–N: 12 mg/*l*). Total N concentration in roots treated with –N medium was significantly lower (22 mg/gDW) than the above treatment. The accumulated N was mainly distributed in a soluble franction of roots, and amount of the insoluble N was not significantly affected by ±N treatments.

When the free amino acid composition was compared between +N and –N treatments (Table 2)[7], glutamine (Gln) was recognized as a major form of the accumulated N in roots with +N medium. On the other hand, in the case of –N treatment, the Gln content was very low and 4-methyleneglutamine (4-MeGln) was a predominant amino acid.

The origin of N accumulated in roots was investigated through supplying ^{15}N labelled medium[12] (Table 3). Approximately 68% of the total N in roots originated from the medium, while the remaining portion of 32% was from the mother bulb. Over 90% of the absorbed labelled N remained in a soluble form, and only a small portion of the absorbed

Table 2. Amino acid composition of 80% ethanol soluble fraction in the roots of tulip plants

Amino acid	+N		-N	
	(μgN/gDW)	(%)[a]	(μgN/gDW)	(%)[a]
Asp	122	0.3	75	1.2
Thr	81	0.2	11	0.2
Ser	409	1.1	16	0.3
Glu	ND[b]	—	177	2.8
Gln	26,542	69.5	266	4.2
4-MeGlu	24	0.06	57	0.9
4-MeGln	6,045	15.8	5,470	87.1
Gly	115	0.3	6	0.1
Ala	515	1.3	8	0.1
Val	99	0.3	22	0.4
Ile	195	0.5	16	0.3
Leu	11	0.03	11	0.2
Tyr	8	0.02	5	0.1
GABA	10	0.03	6	0.1
$EtOHNH_2$	28	0.07	15	0.2
NH_3	200	0.52	36	0.6
Lys	36	0.09	9	0.1
His	132	0.33	24	0.4
Arg	3,644	9.54	52	0.8
Total amino acid-N	38,216	100.0	6,282	100.0

a): Percentage of each amino acid-N in total amino acid-N.
b): ND: Not determined. Glu in the roots of +N was not detected, because the Gln peak was wide and the position of the peak was very near that of the Glu peak.
Source: Ohyama et al. (1985)[7].

Table 3. Distribution of nitrogen in each fraction in the tulip roots originating from the N labelled culture solution and from the bulb

Fraction	Total N content (mgN/gDW)	N from bulb content (mgN/gDW)	Labelled N content (mgN/gDW)	Ratio LN/TN (%)
80% ethanol soluble fraction	28.06	4.64	23.43	83
Water soluble but ethanol insoluble fraction	5.85	1.36	4.49	77
Insoluble fraction	11.31	8.38	2.93	26
Total amount of N	45.22	14.38	30.85	68

Source: Ohyama et al. (1988)[12].

N was assimilated into protein (an insoluble fraction). Based on the ^{15}N abundance of each amino acid, it was confirmed that a major portion of N in free Gln (93%), Ala (87%), Glu (82%), Arg (80%) was derived from the absorbed medium N, whereas only a small portion of N in 4-MeGln (31%) and 4-MeGlu (23%) originated from the absorbed N.

An experiment with split roots was conducted in order to investigatge the movement of accumulated N in those roots during winter[4]. The tulip plants under the experiment had been grown with a -N medium until January, and then a half group of the roots were fed with a ^{15}N labelled culture solution, and another half were put in a -N medium (^{15}N: -N treatment) as well as in a non-labelled +N medium (^{15}N: +N treatment) as shown in Fig. 2. In 5 days after the onset of these treatments, a large amount of N (80 mgN/gDW) was already accumulated in the roots supplied with ^{15}N and +N medium, respectively. However, N content remained constant in

the roots in the –N solution, and ^{15}N was not translocated from the roots with ^{15}N to the opposite side of half roots with the +N or –N medium (Fig. 2). As shown in Table 4, the concentration of Gln increased rapidly and nitrate was detected in the half roots supplied with N. On the other hand, in the opposite side of roots in the –N medium, the level of amino acid was not influenced and NO_3^- was not detected there. Based on the results obtained in the above-noted split-root experiment, it was suggested that the N absorbed in one root be not readily translocated to the other roots. This may mean that each root independently accumulates N after absorption. It is also worthy of notice that tulip roots can absorb N very actively even under a low temperature condition such as 0–5°C.

Fig. 3 shows patterns of incorporation of ^{15}N into free amino acids and amides in due course of time in the roots of tulip plants which were grown under the –N medium[12]. It is obvious that the incorporation of ^{15}N into Gln took place most rapidly among the amino acids detected. The NH_4^+ absorbed or produced by nitrate reduction may be directly assimilated to Gln by a GS/GOGAT system in the roots, and Gln may be simply accumulated in each root. Some part of 4-MeGln could be newly synthesized in the roots from the absorbed medium N, though the turnover rate appeared to be low.

Role of N accumulated in tulip roots during the winter season

The role of N accumulated in the roots for plant growth and bulblets production was investigated[13].

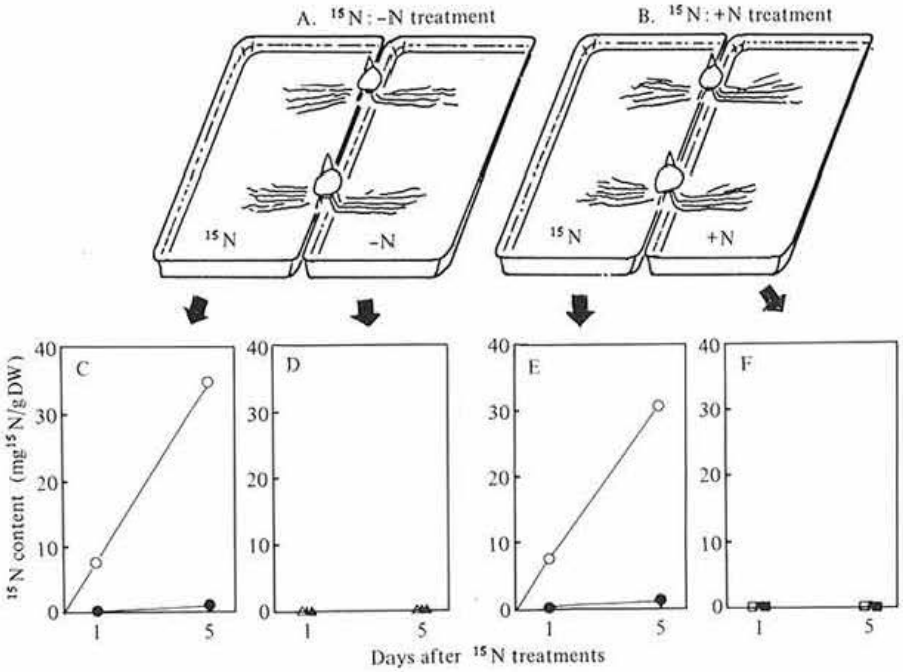

Fig. 2. A root splitting experiment with ^{15}N treatments
A: ^{15}N: –N treatment, B: ^{15}N: +N treatment,
C: ^{15}N incorporation into soluble (○) and insoluble (●) fraction of the roots in ^{15}N medium of ^{15}N: –N treatment,
D: ^{15}N incorporation into soluble (△) and insoluble (▲) fraction of the roots in –N medium of ^{15}N: –N treatment,
E: ^{15}N incorporation into soluble (○) and insoluble (●) fraction of the roots in ^{15}N medium of ^{15}N: +N treatment,
F: ^{15}N incorporation into soluble (□) and insoluble (■) fraction of the roots in +N medium of ^{15}N: +N treatment.
Source: Kitajima et al. (1991)[4].

Table 4. Concentration of N constituents in split roots

(mgN/gDW)

Amino acid	Treated days			
	1		5	
Medium	+N	-N	+N	-N
Asp	0.227	0.262	0.268	0.467
Thr	0.023	—*	0.042	0.039
Ser	0.054	0.033	0.319	0.071
Asn	0.149	0.162	0.625	0.562
Glu	0.248	0.443	0.421	0.873
Gln	2.075	0.337	15.833	0.589
4-MeGln	3.357	6.038	5.278	5.190
Lys	0.293	—	0.348	—
Arg	0.375	0.607	0.995	1.006
Total amino acid-N	6.801	7.882	24.129	8.797
NO_3^--N	3.112	—	10.315	—

* —: Not detected.
Source: Kitajima et al. (1991)[4].

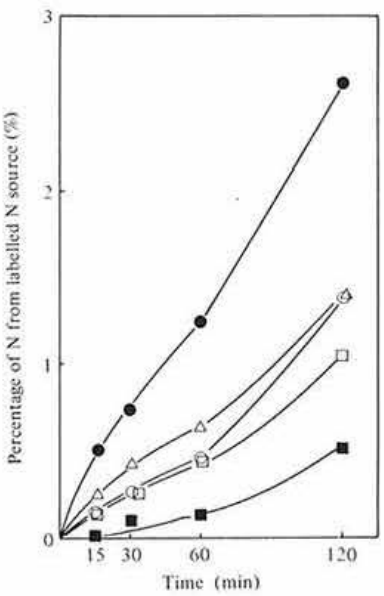

Fig. 3. Patterns of incorporation of ^{15}N into free amino acids and amides in due course of time after feeding in the ^{15}N labelled nutrient solution
●: Gln, ○: Glu, △: Ala, ■: 4-MeGln, □: 4-MeGlu.
Source: Ohyama et al. (1988)[12].

A ^{15}N labelled medium was supplied in order to discriminate the behavior of absorbed N and bulb storage N. As shown in Fig. 4, the patterns of N contents in the shoot growth and bulb production were generally the same with each other for the N originating from a mother bulb and the N accumulated in the roots during winter. After sprouting, both Ns were rapidly translocated to leaves, and finally, about half of those Ns were remobilized to daughter bulbs at harvest. These results suggest that the N absorbed and accumulated in the roots in winter play an important role in supplementing the bulb storage N, and provide a sufficient amount of N for rapid growth of leaves after sprouting.

Accumulation of 4-MeGln and OMPD in vegetative organs

A characteristic amide is 4-MeGln, which is found in tulip and groundnut. The 4-MeGln has been detected in every part of tulip plants, i.e. bulb scales, roots, basal plate, young shoots, leaves, stems and flowers. A high content of 4-MeGln was found especially in leaves, stems and roots after sprouting[7,8,11] (Table 5). Some 22 varieties of tulip analyzed showed that the 4-MeGln contents in leaves and stems were positively correlated with soluble N contents (Fig. 5). These results suggest that 4-MeGln be a temporary N storage compound.

A large quantity of novel organic acid accumulation, i.e. 20–120 mg/gDW was observed in tulip leaves and stems. This acid was isolated and identified as 2-oxo-4-methyl-3-pentene-1,5-dioic acid (OMPD)[9] (Fig. 6). Fig. 7 shows variations in OMPD contents in various organs. The OMPD was greatly accumulated in leaves and stems. It was also

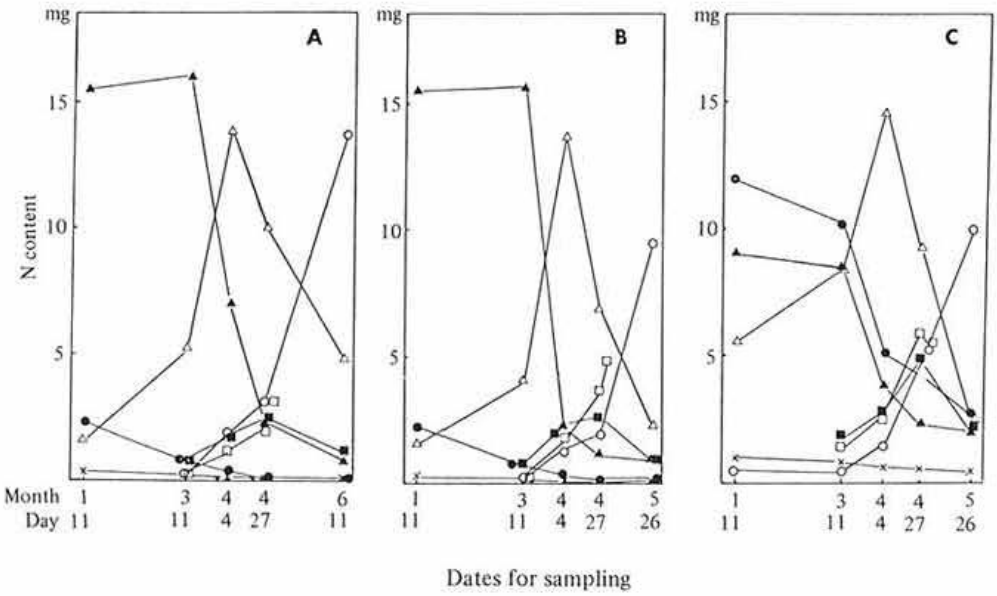

Fig. 4. Contents of labelled N derived from medium and non-labelled N originating from mother bulb scales in various parts of tulip plants

A: Labelled N absorbed from ^{15}N medium, which was applied for 1 month, followed by cultivation under a non-labelled +N medium,

B: Labelled N absorbed from ^{15}N medium, which was applied for 1 month, followed by cultivation under a -N medium,

C: Amount of N originating from bulb storage N, in addition to which ^{15}N labelled medium was applied for 1 month, followed by cultivation under a -N medium.

●: Mother bulb scales, ○: Bulblets, ▲: Roots, △: Leaves, ×: Basal plate, ■: Stem, □: Flower.

Source: Ohyama et al. (1988)[13].

Table 5. Amino acid composition of 80% ethanol soluble fraction in roots and leaves of tulip plants at the flowering stage cultivated in the medium containing nitrogen

Amino acid	Roots		Leaves	
	(μgN/gDW)	(%)[a]	(μgN/gDW)	(%)[a]
Asp	139	0.5	90	0.5
Thr	123	0.4	30	0.2
Glu	260	0.9	197	1.1
Gln	979	3.3	658	3.5
4-MeGln	20,246	67.7	17,184	92.5
Ala	1,425	4.8	107	0.6
Ile	134	0.4	36	0.2
NH_3	57	0.2	54	0.3
Lys	259	0.9	40	0.2
Arg	5,769	19.3	0	0
Total amino acid-N	29,921	100.0	18,578	100.0

a): Percentage of each amino acid-N in total amino acid-N.
Source: Ohyama et al. (1985)[7].

detected in flowers and bulblets, but not in roots, a mother bulb and a basal plate. The OMPD contents in stems increased after sprouting, and rapidly decreased immediately before the flowering stage.

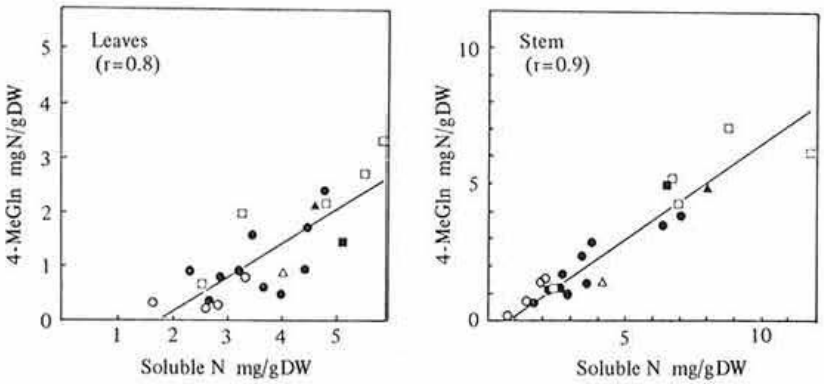

Fig. 5. Relationship between the contents of soluble N and 4-MeGln N in leaves and stems of 22 varieties of tulip
△: Single early tulip, ■: Double early tulip, ●: Mid-season flowering tulip, ○: Single late and parrot tulip, □: Double late tulip and lily-flowered tulip, ▲: Greigii.
Source: Ohyama et al. (1988)[11].

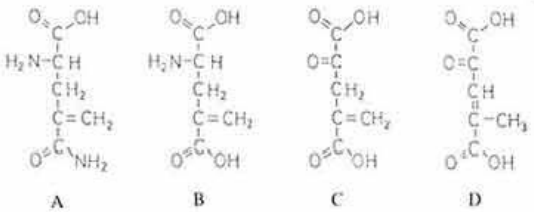

Fig. 6. Structures of 4-MeGln (A), 4-MeGlu (B), 2-oxo-4-methyleneglutamic acid (C) and OMPD (D)
Source: Ohyama et al. (1988)[9].

On the other hand, the leaves continued to accumulate the acid until the flowering stage, and the level did not decrease thereafter until harvesting[9].

The presence of OMPD throughout leaves and stems of *Tulipa gesneriana* was confirmed among 22 varieties, and a positive correlation was observed between the 4-MeGln and the OMPD contents[11]. The physiological role of OMPD is to be identified yet. However, it is very likely that OMPD may be a deamidation and deamination product of the 4-MeGln based on the structural resemblance between them. It is also presumed that the 4-MeGln and OMPD play some other physiological roles, such as the protection from insects or microbes attack.

Long-distance transport of N in tulip plants

In order to pursue a transport form of N from roots to shoots, the basal part of the stem was cut

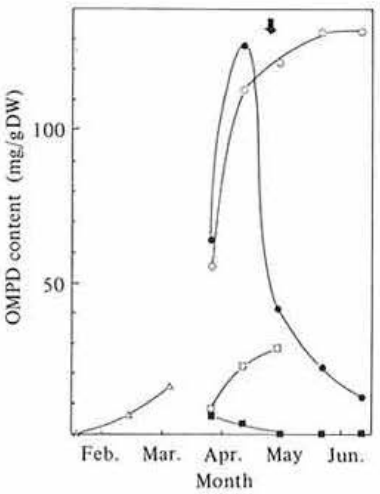

Fig. 7. Variations of OMPD contents in tulip plants in growing period
△: Shoots, ●: Stem, ○: Leaves, □: Flower, ■: Daughter bulbs (bulblets), ⬇: Flowering.
Source: Ohyama et al. (1988)[9].

and the exuded xylem sap was collected. The analysis of N constituents in the sap showed that Gln was a predominant form of N in the xylem fluid. A very small amount of 4-MeGln was detected, but neither 4-MeGln nor NO_3^- was observed. After sprouting, 4-MeGln was a major soluble N constituents in roots, stems and leaves. Therefore, the 4-MeGln seems to be an N storage compound in

these organs, but it is not the transport form of N with a xylem stream. In addition, tulip plants absorb and utilize both NO_3^- and NH_4^+, but a majority of the absorbed NO_3^- appears to be consumed in the roots before it is translocated to shoots. As is the case with the xylem sap, NO_3^- is hardly detectable in leaves and stems of tulip plants.

When a ^{15}N labelled medium was supplied to the plants, the ^{15}N abundance of Gln in xylem sap increased rapidly. Based on these observations, it is tentatively concluded that Gln is a predominant transport form of N in the xylem of tulip plants. It is however presumed that some part of 4-MeGln may be transported from leaves and stems to the sink organs, i.e. flowers and bulblets, via phloem. It was observed that both Gln and 4-MeGln were excreted from the cut basal end of excized leaves and stems. This conclusion has not been fully confirmed yet because of the technical difficulties in obtaining a pure phloem sap.

Fig. 8 summarizes the long-distance transport of N and C in tulip plants. Either NH_4^+ or NO_3^- absorbed from the culture medium may be initially assimilated into Gln via a GS/GOGAT system in the roots. During the winter season, the Gln is accumulated in the roots before sprouting. After sprouting, the major part of the Gln synthesized is immediately transported to shoots with a xylem stream. Some part of N is used for 4-MeGln synthesis in the roots. The transported Gln in shoots are utilized for the protein and 4-MeGln synthesis.

Fig. 9 schematically shows C and N transport via phloem. The photosynthetic product is transported from leaves to the other parts mainly in the form of sucrose (Suc). The Suc provided from leaves supports the growth and metabolism of non-photosynthetic organs. Furthermore, the Suc transported to the bulblets are stored in the form of starch granules. The starch is converted to Suc and

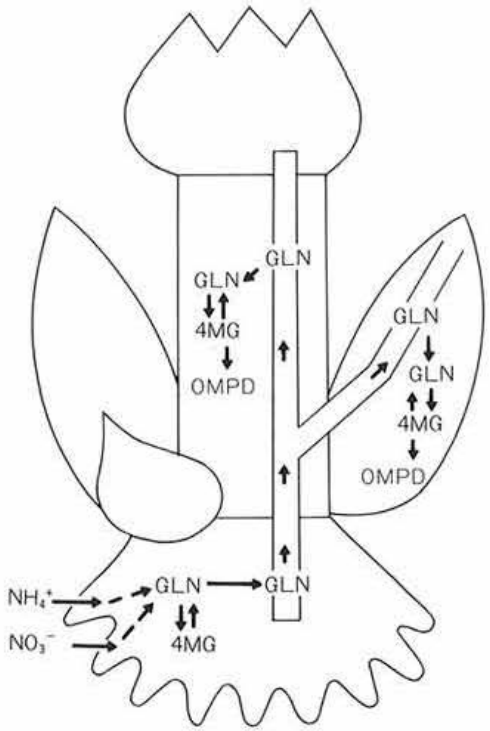

Fig. 8. Schematic N-flows in xylem of tulip plants
GLN: Glutamine, 4MG: 4-Methyleneglutamine, OMPD: 2-oxo-4-methyl-3-pentene-1,5-dioic acid.
Source: Unpublished.

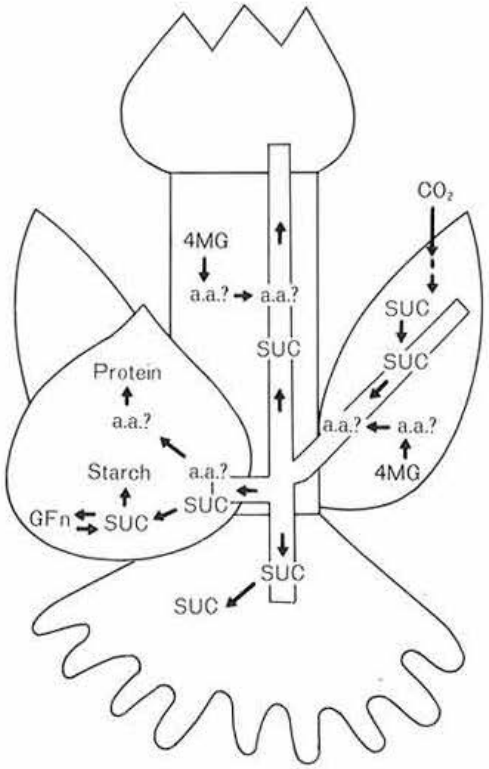

Fig. 9. Schematic C- and N-flows in phloem of tulip plants
SUC: Sucrose,
GFn: Fructosylsucroses (DP = n + 1),
a.a.: Amino acids.
Source: Unpublished.

fructosylsucroses, if the bulbs are exposed to the low temperature. It then provides the C skeletons with energy and reducing power for root development and N accumulation during the underground existence in winter[10,14]. The form of N transport via phloem has to be confirmed yet. Gln and 4-MeGln might be potential candidates for a phloem N transporter. During the maturing stage, a large portion of N in the vegetative parts is redistributed to the bulblets and used for synthesis of storage protein in bulb scales. It was found that the storage protein decomposition is also induced by a low temperature, and the protein N is convertd to soluble N especially Gln in bulb scales during the winter season[10].

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