Nitrate and Nitrite Are Transported by Different Specific Transport Systems and by a Bispecific Transporter in Chlamydomonas reinhardtii*

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**Nitrate transport mutants from Chlamydomonas reinhardtii and strains derived from them upon transformation with plasmids containing the C. reinhardtii nar2/Nrt2;1 or nar2/Nrt2;2 genes have been used to study nitrate and nitrite transport systems. Mutants lacking nitrate assimilation clustered genes showed a high affinity nitrite transporter activity (system 3), which was subject to ammonium inhibition and appeared to be independent of a functional nar2 gene. Transformants carrying nar2/Nrt2;2 recovered a high affinity nitrate transporter activity (system 2) and showed nitrite transport activities with properties similar to those in non-transformed cells. Transformants carrying nar2/Nrt2;1 recovered high affinity nitrate transporter activity (system 1) together with a considerably enhanced nitrite transport activity. Nitrite transport mediated by system 1 was very sensitive to inhibition by nitrate at μM concentrations. Results strongly suggest that three nitrate assimilation related high affinity transport systems operate in C. reinhardtii: one specific for nitrite, a second one encoded by nar2/Nrt2;2 specific for nitrate, and another one encoded by nar2/Nrt2;1, which is bispecific for these two anions.

Nitrate, the preferred nitrogen source in photosynthetic organisms, is assimilated by a highly regulated pathway whose first step is the entry of nitrate into the cells mediated by an active transport system (1–3). In plant cells, it is believed that at least two transport systems are involved in nitrate uptake. One is a high affinity system that is nitrate inducible (4–6) and another one is a low affinity system that is constitutively expressed (7). An intensive effort has recently been addressed to the isolation and characterization of the corresponding genes, since nitrate transport controls the amount of nitrogen assimilated by the cells (3).

Nitrate transporter (NT)1 genes (nrtA, -B, -C, -D) have been cloned in both unicellular and filamentous cyanobacteria and correspond to typical bacterial binding protein-dependent transport systems (8–11). The crnA gene from the filamentous fungus Aspergillus nidulans has been proposed to encode for a nitrate transporter (12, 13). nar2, Nrt2;1, and Nrt2;2 from the green alga Chlamydomonas reinhardtii have recently been identified as high affinity NT genes (14, 15). Nrt2;1 and Nrt2;2 were formerly named nar3 and nar4, respectively, and their new names will be used hereafter to meet plant gene nomenclature recommendations of the International Plant Molecular Biology Society (16). The Arabidopsis thaliana chl1 (Nrt1) gene is proposed to encode a low affinity NT (7, 17) and does not show significant homology with any of the above-mentioned genes encoding high affinity NT (10, 15).

In C. reinhardtii, genes related to nitrate transport (nar2, Nrt2;1, and Nrt2;2) are located within a nitrate-regulated gene cluster containing the NR structural gene Nia1 (14, 15, 18). Mutants deleted in the nar2-NRT2;2 genomic region are true NT mutants, which recover a high affinity NT activity upon transformation with plasmids carrying either nar2 plus Nrt2;1 or nar2 plus Nrt2;2 but not with any of these genes separately (15). NRT2;1 and NRT2;2 proteins show a highly significant identity at the amino acid level with each other and with CRNA from A. nidulans (13) but not with any of the cyanobacterial nrt genes (10, 11). CRNA, NRT2;1, and NRT2;2 are hydrophobic proteins that appear to have 12 hydrophobic membrane-spanning domains that would form the channel for nitrate (13, 15). It has been suggested that NAR2 is either a structural or regulatory protein required for the function of NRT2;1 and NRT2;2, which could represent two alternative NT systems (15).

In this work, NT mutants and transformed strains carrying different sets of nar genes have been molecularly and functionally characterized. Data strongly suggest that in C. reinhardtii there exist a nitrite-specific transporter independent of nar2 (system 3) and two additional NT systems: NAR2/NRT2;2 (system 2), which is specific for nitrate, and NAR2/NRT2;1 (system 1), which efficiently transports both nitrate and nitrite.

**EXPERIMENTAL PROCEDURES**

Strains and Growth Conditions—C. reinhardtii wild type 21gr mt+ and the NT mutant S16 mt−, possessing a functional NR gene integrated at a locus unlinked to the nitrate gene cluster, have been characterized elsewhere (15, 19). Strains 63–2 and 63–4 have been obtained by transformation of S16 mutant with plasmid pT2 carrying the nar2 and Nrt2;1 genes. Strains 65–3 and 65–4 have been obtained by transformation of S16 strain with plasmids pB6a and pT3 carrying nar2 and Nrt2;2, respectively (Fig. 1, and Ref. 15).

Cells were grown at 25°C under continuous light with 3–5% CO2-enriched air in ammonium minimal HS medium (20). Cells, induced in 4 mM KNO3 media for 3 h (15), were thoroughly washed and used for transport experiments.

Genetic Crosses—Genetic analyses were carried out by the random spore plating method according to Levine and Ebersold (21), as modified by Van Winkle-Swift (22). Other details and media were as described previously (23).

DNA Isolation from C. reinhardtii and Hybridization Analysis—Genomic DNA isolation and Southern transfer analysis were performed as described by Ranum et al. (24). Fractionated DNA was transferred to
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Southern analysis of genomic DNA from the NT mutant S16 and transformant strains. A, restriction map of the genomic DNA region containing the nitrate assimilation gene cluster. Enzymes used are H, HindIII; K, KpnI; S, SstI; Sa, SalI; and Sm, SmaI. Approximate location and transcriptional orientation of nar, Nrt2, and Nia1 genes is shown by solid arrows (15). C, reinhardtii DNA in plasmids pT1, pT2, and pB6a is indicated above the map. Probes B6a-6.2, a Sall-SstI genomic fragment, and 3′n4, a 360-bp pair HindIII-EcoRV fragment at the 3′-non-translated region of Nrt2;2, are indicated above the map. Probes B6a-6.2, a 360-base-pair HindIII-EcoRV fragment at the 3′-non-translated region of Nrt2;2, cDNA, are indicated by solid lines. a, b, and c indicate expected fragments hybridizing these probes in Southern blots of genomic DNA digested with the indicated enzymes. B, genomic DNA (2–3 μg) isolated from the strains S16 (lane 1), 63–2 (lane 2), 63–4 (lane 3), 65–3 (lanes 4 and 6), and 65–4 (lanes 5 and 7) was digested with HindIII (lanes 1–3), SstI (lanes 4 and 5), and Smal + KpnI (lanes 6 and 7) and analyzed by Southern hybridization with the indicated probes. C, genomic DNA (2–3 μg) isolated from strains S16 (lanes 1 and 5), 63–2 (lanes 4 and 6), 63–4 (lanes 5 and 7), 65–3 (lanes 2 and 9), and 65–4 (lanes 3 and 10) was digested with KpnI (lanes 1–5) and Smal (lanes 6–10) and analyzed by Southern hybridization with the indicated probes.

nylon membranes (Hybond-N, Amersham) according to the instructions of the manufacturers. Radioactive probes were labeled by the random primer method (25).

Nitrate and Nitrate Uptake—Nitrite uptake was determined under conditions of temperature, light, and CO2 indicated above in media containing 50 mM Tris-HCl buffer, pH 7.5, 20 mM KCl, 10–100 mM KNO2, and nitrate-induced cells (10–30 μg of chlorophyll/ml). Samples were taken at 1–2 min intervals, spun down in a microfuge, and nitrite was determined in the supernatant medium. Nitrate uptake was measured under the same conditions as for nitrite uptake except that KNO2 was replaced by 20–100 mM KNO3.

Analytical Methods—Nitrite was measured colorimetrically according to Snell and Snell (26). Nitrate was determined by ion exchange high pressure liquid chromatography using a Nucleosil 100–10S5 10-μm column (250 × 4 mm) equilibrated with 125 mM potassium phosphate buffer, pH 4, at a flow rate of 3 ml/min. Nitrate was detected using a UV detector at 210 nm (15). Chlorophyll was determined according to Arnon (27).

**RESULTS**

Strains Carrying Separately Each of the NT Systems—The NT mutant strain S16 has been complemented for growth on 2 mM nitrate media by transformation with either plasmid pT2 containing nar2 and Nrt2;1 or plasmids pB6a plus pT1 containing nar2 and Nrt2;2, respectively (Ref. 15 and Fig. 1A). Each of the NAR2/NRT2;1 or NAR2/NRT2;2 proteins are referred hereafter as NT system 1 and 2, respectively. Several transformants were taken for further characterization: strains 63–2 and 63–4 (carrying system 1) and strains 65–3 and 65–4 (carrying system 2). Southern blots of genomic DNA digests of S16 showed no hybridization bands with the 3′n4 and B6a-6.2 probes, since this mutant is deleted within this region (Fig. 1B, lane 1; Fig. 1C, lanes 1 and 8). Completeness of the DNA introduced by transformation was demonstrated by digestion with enzymes that release fragments containing the functional genetic markers (Fig. 1B). In strains 63–2 and 63–4, transformed with plasmid pT2, bands of 8.5 and 7 kb, respectively, were obtained in blots of HindIII digests probed with B6a-6.2 (Fig. 1B, lanes 2 and 3). The smaller size for this band than expected (8.5 kb) in strain 63–4 indicates that random insertion of pT2 in the genome of this strain took place within this HindIII-HindII fragment. Strains 65–3 and 65–4 were transformed with the two plasmids pB6a and pT1 together. Intactness of pB6a DNA containing a functional nar2 gene was studied by SstI digestion, which should produce a 4-kb band detectable with the B6a-6.2 probe. In both strains, a band of this size was obtained, but an additional band of 5 kb was detected for strain 65–3 (Fig. 1B, lanes 4 and 5), indicating that an extra copy of this pB6a DNA was also present. The existence of pT1 carrying a functional Nrt2;2 gene in transformants 65–3 and 65–4 was studied from Smal + KpnI DNA digestions and hybridization with the 3′n4 probe. A 3-kb band was detected for both transformants as expected (Fig. 1B, lanes 6 and 7).

The copy number of introduced DNA in transformants was studied by digestions with enzymes that allow detection of bands with a restriction site in the genomic boundaries of the inserted plasmid DNA (Fig. 1C). Genomic DNA of strains 63–2 and 63–4 was digested with KpnI or Smal and hybridized to the B6a-6.2 probe rendering unique bands of different sizes (lanes 4–7). This suggests that a single copy of pT2 integrated at different positions in the genome of each transformant. The pB6a flanking region was detected in 65–3 and 65–4 strains from KpnI digestions and hybridization with B6a-6.2. As shown in Fig. 1C, two hybridization bands were detected for transformant 65–3 and only one for 65–4 (lanes 2 and 3, respectively), which indicates again the presence of two copies of this B6a region in strain 65–3. The copy number of pT1 was studied by Smal digestions of 65–3 and 65–4 genomic DNA and hybridization with the 3′n4 probe. Interestingly, two bands were detected for the 65–3 DNA and only one for 65–4 (Fig. 1C, lanes 9 and 10), which suggests that two copies of pT1 DNA are also present in the 65–3 genome.

On the other hand, the stability through meiosis of the newly introduced genetic markers in the transformant strains was studied by genetic crosses with the wild-type 21gr strain (Table 1), considering that the lack of either of nar2, Nrt2;1, Nrt2;2 or Nia1 leads to a Nia− phenotype (inability to grow on nitrate minimal medium) (15). Segregation ratio Nia+:Nia− in the different crosses was very close to 2:1. A segregation 9:7 should be found for un linkage among all the studied genes and 3:1 for linkage among all. The ratio found is close to 5:3, which would
be the expected one for the two nar2 and Nrt2 genes linked to each other and unlinked to Nia1 and the nitrate cluster region. In fact, nar2 and Nrt2;1 genes are physically linked in transformants with pT2 (strains 63–2 and 63–4), and it is known that Nia1 is unlinked to the gene cluster region in the parental strain S16 (15). In addition, a loss of viability cannot be ruled out in the segregations, since the S16 mutant was isolated from Nrt2; strain C2, which has a long deletion, and DNA rearrangements occur in these mutant strains selected with chlorate (14). To study the existence of linkage in the newly introduced DNA, four segregants from the cross 65–3 × 21gr and two from 65–4 × 21gr, having a Nia1– phenotype and an active NR enzyme, were selected. Southern blot analysis of their genomic DNA showed that pT1 and pB6a plasmids were absent (results not shown), which suggests that both genetic markers are linked. It has been shown that plasmids integrated in several copies in C. reinhardtii tend to be genetically linked (28).

Characterization of NiT and NT Activities in the NT-deficient Mutant S16 and Transformants Carrying Systems 1 or 2—The parental strain S16 (Δnar2, Nrt2;1, and Nrt2;2) grows well in nitrite medium and shows a nitrite uptake activity at low nitrite concentrations, 25 μM and less. This activity was completely blocked by ammonium (Fig. 2). Since S16 cannot take up nitrate, this result indicates that a specific NiT system is present in this strain. Integration of the nar2 and Nrt2;1 genes in S16 (strains 63–4 and 63–2) led to four to five times increased nitrite uptake rates (Fig. 2), which suggests that the NT system 1 mediates the entrance of nitrite into the cell even more efficiently than the specific NiT system 3 described above. However, integration of nar2 and Nrt2;2 in S16 (strains 65–3 and 65–4) resulted in nitrite uptake rates very similar to those in the parental strain S16 (Fig. 2). This result suggests that NT system 2 encoded is specific for nitrate and does not efficiently transport nitrite.

Kinetic characterization of nitrite uptake and inhibition by nitrate has been studied in the C. reinhardtii wild type strain (29). The results indicated that nitrate is a partially competitive inhibitor of nitrite uptake and seem to fit with the presence of different transporters for nitrate and nitrite in C. reinhardtii. Competition by nitrate and its analogue chlorate (30) on the nitrite transport by strains S16, 63–2, 63–4, 65–3, and 65–4 has been studied. As shown in Fig. 3, neither nitrate (50 μM) nor chlorate (1.5 mM) had an effect on nitrite uptake rates in strains S16 (carrying system 3) and 65–3 or 65–4 (carrying systems 2 and 3). However, both ions inhibited significantly nitrite uptake in strains 63–2 and 63–4 (carrying systems 1 and 3).

The nitrite uptake inhibition by nitrate was also studied in the above strains by analyzing the effect of increasing nitrate concentrations on nitrite uptake, according to Córdoba et al. (29). Data in Fig. 4 show that nitrate concentrations up to 100 μM did not significantly affect nitrite uptake rates of system 3 determined at 10, 25, 50, and 75 μM nitrite in S16, 65–3, and 65–4 strains. In contrast, nitrate at μM concentrations strongly inhibited nitrite uptake rates of system 1 in strains 63–4 and 63–2. Analysis of these data are consistent with the existence of different transporters for nitrate and nitrite in C. reinhardtii (29).

Kinetic parameters for nitrate and nitrite transport in these strains have been determined (Table II). In S16 strain, which only transports nitrite by system 3, Ks for NiT was 3.4 μM, the same value as in strain 65–3, which has in addition the NT system 2. In this strain, 65–3 nitrate was transported with a Ks
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A protein carrier specific for nitrite is involved in nitrite transport in the NT mutant strain S16, with an apparent Ks of 3.4 μM. This NT activity seems not to be due to a passive influx of nitrite since no diffusion component was found under our experimental conditions (Ref. 29 and this work). Nitrite uptake in C. reinhardtii was also blocked by ammonium in contrast to the cyanobacterial passive nitrite transport, which is insensitive to ammonium (31).

The short-term blocking by ammonium of the nitrate/nitrite uptake is a well studied effect in C. reinhardtii and other organisms (1, 31–33). However, its precise mechanism is still unknown. The inhibition is typically reversible, so the ability of the cell to utilize nitrate/nitrite is restored after ammonium is consumed from the medium. Both ammonium itself and a (some) product(s) of its metabolism seem to act together to block nitrite uptake in C. reinhardtii (33).

The protein carriers for NT identified in C. reinhardtii are encoded by the crnA-homologous genes Nrt2;1 and Nrt2;2, with the requirement of a functional nar2 gene (15). System 1 (NAR2/NRT2;1) appears to mediate a high affinity and a high capacity transport specific for both nitrate and nitrite, whereas system 2 (NAR2/NRT2;2) mediates a still high affinity and specific NT, which showed a lower affinity and capacity for nitrate than system 1. In C. reinhardtii, chlorate sensitivity depends on chlorate concentration (30). Our results support that sensitivity to chlorate 1.5 mM is mostly mediated by system 1. In Synechococcus sp. PCC 7942, nrtA, nrtB, nrtC, and nrtD are responsible for a multicomponent transporter system that mediates transport of both nitrate and nitrite (34). In higher plants, nitrate transport seems to be a more complex process; a biphasic nitrate uptake kinetics has been reported, so that constitutive and inducible systems might account for nitrate transport (5–7, 17, 35). In addition, the existence of specific nitrite transporters is uncertain (2, 3). The clustered Nrt2/nar genes are ammonium-repressible and nitrate-inducible (34), their expression requires an active product of the regulatory gene nit2 (14, 37), and according to our data they encode for high affinity transporters specific for nitrate and nitrite. The role of nar2 gene product seems to be specific for the NT systems 1 and 2, since the lack of a functional nar2 gene appears to have no effect on the regulation of the Nia1 gene other than the lack of nitrate induction because of a deficient NT activity (15). The gene(s) responsible for the specific nitrite transport in C. reinhardtii, which is functional in strain S16, has not yet been isolated.

Nrt2;1 transcripts accumulate under different nutritional and physiological conditions in amounts much higher than those of Nrt2;2, and their amounts respond differentially to the culture growth phase (36). In this context, it is worth pointing out that the Vmax found for the three transport systems studied reflect how efficiently the corresponding system has been induced. In S16, unable to take up nitrate, nitrate induction of the ammonium-repressible system 3 specific for nitrite is not as efficient as in strains carrying also the nitrate-specific transporter system 2. Similar Vmax values were determined for two strains carrying a different copy number of the nar2 and Nrt2;2 genes (molecular analysis suggests the presence of two copies of pT1 and pB6a in 65–3, but only one in 65–4). If both copies are functional, this might reflect a posttranscriptional control of the nitrate transport machinery in C. reinhardtii. The response of these transporters to nutritional signals for adjusting their relative amounts in the cell and their specific role in promoting an efficient nitrate and nitrite transport are still open questions.

**FIG. 4.** Effect of different nitrate concentrations on the nitrite uptake rate by S16, 63–2, 63–4, 65–3, and 65–4 strains. Initial nitrite uptake rates were determined at the KNO3 concentrations indicated in abscissa, in the absence of nitrate (○) or in the presence of nitrate (10 μM, □; 25 μM, △; 50 μM, ▲; or 100 μM, ■).

**TABLE II** Kinetic parameters of nitrate and nitrite transporters in C. reinhardtii.

Data are from nitrate-induced cells. Kinetic parameters are as determined by Córdoba et al. (29).

| Strains       | Nitrate       | Nitrite       |
|---------------|---------------|---------------|
|               | Ks (μM) | Vmax (μmol/h/mg chlorophyll) | Ks (μM) | Vmax (μmol/h/mg chlorophyll) |
| S16 (system 3)| 3.4 ± 0.4 | 2.6 ± 0.3     | 9.4 ± 2.2 | 1.6 ± 0.6 | 9.0 ± 1.1 |
| 63-4 (systems 1+3) | 1.8 ± 0.3 | 5.2 ± 2.4  | 11.0 ± 1.3 | 5.6 ± 1.5 |
| 65-3 (systems 2+3) | 3.3 ± 0.6 | 10.0 ± 0.6a | 1.1 ± 0.1 | 11.0 ± 1.7 |

*Data from Córdoba et al. (29).*

of 11 μM. In strain 63–4 carrying system 1, Ks values were the lowest of studied strains, 1.8 μM for nitrite and 1.6 μM for nitrate. Highest values of Vmax were also obtained in strain 63–4. Kinetic parameters obtained for nitrate (29) and nitrate transport (Table II) in the wild type strain were very similar to those obtained in strain 63–4. Vmax for NIT in strain 65–3 was higher than in S16. This reflects a better expression of the nitrite consuming system 3 in strain 65–3 during nitrate induction, since the lack of NT activity in S16 prevents an efficient induction of the system 3. In fact, by increasing nitrate concentrations in the medium to 50 mM and higher, an induction of system 3 comparable to that in the strains also carrying system 2 was achieved (not shown).

**DISCUSSION**

Data in this work strongly support a model in which three different nitrate/nitrite transport systems operate in C. reinhardtii. A protein carrier specific for nitrite is involved in nitrite transport in the NT mutant strain S16, with an apparent Ks of 3.4 μM. This NT activity seems not to be due to a passive influx of nitrite since no diffusion component was found under our experimental conditions (Ref. 29 and this work). Nitrite uptake in C. reinhardtii was also blocked by ammonium in contrast to the cyanobacterial passive nitrite transport, which is insensitive to ammonium (31).

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REFERENCES

1. Guerrero, M. G., Vega, J. M., and Losada, M. (1981) Annu. Rev. Plant Physiol. 32, 169–204
2. Crawford, N. M., and Arst, H. N., Jr. (1993) Annu. Rev. Genet. 27, 115–146
3. Hoff, T., Troung, H. N., and Caboche, M. (1994) Plant Cell Environ. 17, 489–506
4. Hole, D. J., Emran, A. M., Fares, Y., and Drew, M. C. S. (1990) Plant Physiol. 93, 642–647
5. Siddiqi, M. Y., Glass, A. M. D., Ruth, T. J., and Rufty, T. W. (1990) Plant Physiol. 93, 1426–1432
6. Glass, A. D. M., Shalf, J. E., and Kochian, L. V. (1992) Plant Physiol. 99, 456–463
7. Doddema, H., and Telkamp, G. P. (1979) Physiol. Plant. 45, 332–338
8. Omata, T. (1991) Plant Cell Physiol. 32, 151–157
9. Omata, T., Ohnori, M., Arai, N., and Ogawa, T. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6612–6616
10. Omata, T., Andriesse, X., and Hirano, A. (1993) Mol. & Gen. Genet. 236, 193–202
11. Merchán, F., Kindle, K. L., Llama, M. J., Serra, J. L., and Fernández, E. (1995) Plant Mol. Biol. 28, 759–766
12. Johnston, I. L., McCabe, P. C., Greaves, P., Gurr, S. J., Cole, G. E., Brow, M. A. D., Unkles, S. E., Clutterbuck, A. J., Kinghorn, J. R., and Innis, M. A. (1990) Gene (Amst.) 90, 181–192
13. Unkles, S. E., Hawker, K. L., Grieve, C., Campbell, E. I., Montague, P., and Kinghorn, J. R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 204–208
14. Quesada, A., Galván, A., Schnell, R. A., Lefebvre, P. A., and Fernández, E. (1993) Mol. & Gen. Genet. 240, 387–394
15. Quesada, A., Galván, A., and Fernández, E. (1994) Plant J. 5, 407–419
16. Caboche, M., Campbell, W., Crawford, N. M., Fernández, E., Kleinhofs, A., Ida, S., Mendel, R., Omata, T., Rothstein, S., and Wray, J. (1994) Plant Mol. Biol. Rep. 12, 45–49
17. Tsay, Y. F., Schroeder, J. I., Feldmann, K. A., and Crawford, N. M. (1993) Cell 72, 705–713
18. Fernández, E., Schnell, R., Ranum, L. P. W., Hussey, S. C., Silflow, C. D., and Lefebvre, P. A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6449–6453
19. Fernández, E., and Matagene, R. (1984) Curr. Genet. 8, 635–640
20. Suesse, N., Chiang, K. S., and Kates, J. R. (1967) J. Mol. Biol. 25, 47–66
21. Levine, R. P., and Ebersold, W. T. (1960) Annu. Rev. Microbiol. 14, 197–216
22. Van Winkle-Swift, K. P. (1997) J. Physiol. 13, 225–231
23. Harris, E. (1989) A Chlamydomonas Sourcebook, pp. 419–427, Academic Press, New York
24. Ranum, L. P. W., Thompson, M. D., Schloss, J. A., Lefebvre, P. A., and Silflow, C. D. (1987) Genetics 120, 109–122
25. Feinberg, A. D., and Vogelstein, B. (1984) Anal. Biochem. 137, 266–267
26. Snell, F. D., and Snell, C. T. (1949) Colorimetric Methods of Analysis, Vol. 2, pp. 804–807, Van Nostrand, New York
27. Arnon, D. I. (1949) Plant Physiol. 24, 1–15
28. Kindl, K. L., Schnell, R. A., Fernández, E., and Lefebvre, P. A. (1989) J. Cell Biol. 109, 2589–2601
29. Córdoba, F., Cárdenas, J., and Fernández, E. (1986) Plant Physiol. 82, 904–908
30. Prieto, R., and Fernández, E. (1993) Mol. & Gen. Genet. 237, 429–438
31. Flores, E., Herrero, A., and Guerrero, M. G. (1987) Biochim. Biophys. Acta 986, 103–108
32. Flores, F. J., and Vega, J. M. (1983) Planta 158, 288–293
33. Córdoba, F., Cárdenas, J., and Fernández, E. (1987) Biochim. Biophys. Acta 902, 287–292
34. Luque, I., Flores, E., and Herrero, A. (1994) Biochim. Biophys. Acta 1184, 296–298
35. Watt, D. A., Amory, A. M., and Cresswell, C. F. (1992) J. Exp. Bot. 43, 605–615
36. Quesada, A., and Fernández, E. (1994) Plant Mol. Biol. 24, 185–194
37. Galván, A., Cárdenas, J., and Fernández, E. (1992) Plant Physiol. 98, 422–426
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