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Trehalose Study Medicinal Properties Isolated from Tobacco Plants and Treatment of α-Crystallin Diseases

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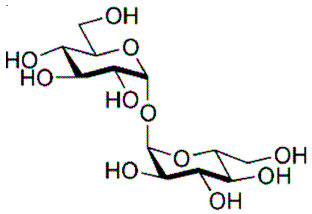
Tobacco is an agricultural product processed from the leaves of plants in the genus Nicotiana. It can be consumed, used as an organic pesticide and, in the form of nicotine tartrate, used in some medicines. Trehalose, a simple disaccharide, has been shown to be effective in preventing the deactivation of numerous proteins and in protecting cells against stress. This study and analysis of trehalose from leaves of *Nicotiana tobacum* show some diseases that will be better with using by trehalose. In this study, tobacco (*Nicotiana tobacum* L.C.V E1) was analyzed for the presence of trehalose. Using gas chromatography-mass spectrometry (GC-MS) analysis, trehalose was unambiguously identified in extracts from leaves *Nicotiana tabacum*. So, it was found that trehalose had accumulated in leaves.

Key Words: Trehalose, α-Crystallin, Alzheimer's disease, *Nicotiana tabacum*, GC-MS.

INTRODUCTION

Plants sense and respond to changes in carbon and nitrogen metabolites during development and growth according to the internal needs of their metabolism. Sugar-sensing allows plants to switch off photosynthesis when carbohydrates are abundant.

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyrano-side) is in bacteria, yeast cells, fungal spores, certain invertebrate species and resurrection plants and animals that it is non-reducing disaccharide present[1-8]. It can serve as reserve of carbohydrate and as a protestant in response to different stress such as dehydration, salinity, freezing and heat stress. It may also serve as a source of energy and carbon[9,10].



Trehalose

The best known biosynthesis pathway of trehalose included two steps: (1) Trehalose-6-phosphate is first formed from uridine diphosphate-glucose. (2) Glucose-6-phosphate in a reaction catalyzed by trehalose-phosphate synthase. Trehalose-6-phosphate is then converted to trehalose by trehalose-phosphate phosphatase (TPP). Each trehalose-phosphate synthase and trehalose-phosphate phosphatase plays an important role in the regulation of trehalose production for the organism growth and development[11]. Trehalose not only acts as a carbohydrate reserve but also has higher water retention ability. It can stabilize dehydrated enzymes, proteins and lipid membranes efficiently, as well as protect biological structures from damage during desiccation in bacteria, yeast, fungi and invertebrates[11-13]. In plants, on the contrary, only other sugars and compatible solutes were accumulated under a biotic stresses and trehalose has not been detected in previous studies. Most plants are thought to lack the capacity to synthesize trehalose [14]. Recently, trehalase genes have been found in soybean (glycine max) and arabidopsis[15-17]. Also trehalase activity has been detected in a wide range of higher plants[18]. After inhibition of trehalase, trehalose synthesis can be detected in arabidopsis[16]. Therefore, the apparent lack of trehalose accumulation in plants was probably due to the presence of trehalase activity.

Tobacco is an agricultural product processed from the leaves of plants in the genus Nicotiana. It can be consumed, used as an organic pesticide and, in the form of nicotine tartrate, used in some medicines[19]. It is most commonly used as a recreational drug and is a valuable cash crop for countries such as Cuba, China and the United States.

Nicotiana tobacum, or cultivated tobacco, is a perennial herbaceous plant. It is found only in cultivation, where it is the most commonly grown of all plants in the Nicotiana genus and its leaves are commercially grown in many countries to

be processed into tobacco. It grows to heights between 1 to 2 m. Leaves are very varied in size, the lower leaves are the largest up to 60 cm long, shortly stalked or unstalked, oblonged-elliptic, shortly acuminate at the apex, recurrent at the base, the following leaves decrease in size, the upper one sessile and smallest, oblong- lanceolate or elliptic.

Every part of the plant except the seed contains nicotine, but the concentration is related to different factors such as species, type of land, culture and weather conditions. The concentration of nicotine increases with the age of the plant. Tobacco leaves contain 2 to 8 % nicotine combined as malate or citrate. The distribution of the nicotine in the mature plant is widely variable: 64 % of the total nicotine exists in the leaves, 18 % in the stem, 13 % in the root and 5 % in the flowers. Tobacco has been used as an antispasmodic, a diuretic, an emetic, an expectorant, a sedative and a sialagogue and in homeopathy.

Tobacco has a long history of use by medical herbalists as a relaxant, though since it is a highly addictive drug it is seldom employed internally or externally at present. The leaves act as antispasmodics, dissentients, diuretics, emetics, expectorants, irritants, sedatives and sialagogues. They are used externally in the treatment of rheumatic swelling, skin diseases and scorpion stings. The plant should be used with great caution, when taken internally it is addictive. The active ingredients can also be absorbed through the skin. Wet tobacco leaves can be applied to stings in order to relieve the pain. They are also a certain cure for painful piles. A homeopathic remedy is made from the dried leaves. It is used in the treatment of nausea and travel sickness. Some other activities reported for *Nicotiana tobacum* are: analgesic activity, anesthetic activity, angiogenesis inhibition, antibacterial activity, anti convulsant activities, anti estrogenic effect, antifungal activity, antiglaucomic activity, antioxidant activity, antistress effect, antiviral activity, aromatase inhibition, arrhythmogenic effect, carcinogenic activity, bronchoconstrictor activity, bupivacaine kinetics.

Most of the tobacco grown in large western mainly in three northern provinces (Gilan, Mazandaran, Golestan) of Iran and an area of over eight thousand acres in the covers. For farmers, this type of tobacco grown in these areas is very important and the best type of tobacco product in terms of amount and quality are considered[20]. Essential element in plant growth micro tobacco and tobacco is one plant is like the accumulation of chlorine in the chlorine quickly and too much can be done, however large accumulation of this element on the quality of the leaves of this plant puts undesirable[21]. Toxicity resulting from chlorine concentration, in addition to reducing the quality of tobacco plants decreased dry weight leaf, height and yield of tobacco products and also change the membrane permeability, chlorophyll content of leaves, change the lipid membrane and flowering time can be changed[22].

The role of trehalose in α-crystalline stress tolerance, chaperone activity and thermal stability is studied. That crystalline is the predominant protein within the eye lens[23]. Since trehalose protects and stabilizes the cell membrane; it has been used as a preservative for tissues in organ transplantation[24]. Indental research, that exposure of intra-oral and tooth surfaces to trehalose exhibits a slower pH response than exposure to sucrose[25]. So, trehalose undergoes less acid fermentation and production by streptococci mutants and rat dental caries are reduced by the presence of dietary trehalose[25]. But trehalose is not an antioxidant and has been shown to have a protective effect on the deleterious effects of low temperature on the membranes of ram sperm[26] and dog sperm[27].

Alzheimer's disease (AD) is a progressive dementia and the number of patients increases exponentially with life expectancy[28]. The development of an effective inhibitor of Alzheimer's disease is urgently required. Alzheimer's disease is characterized neuropathologically by extracellular deposition of amyloid senile plaques and neurofibrillary tangles in vulnerable Alzheimer's disease brain regions. These plaques are primarily composed of fibrils of the amyloid β (Aβ) peptide, a small peptide composed of 39-43 amino acids[29]. The most abundant forms are 40 and 42 amino acids in length[30,31]. These types of peptide are cleaved from a large protein called the amyloid precursor protein (APP) by a secretase enzyme[32]. Production of Aβs is a normal occurrence, although its function remains unclarified. However, in Alzheimer's disease patients the peptide forms ordered fibrillar aggregates[33]. Therefore, it is required to develop medicinal compounds that inhibit Aβ aggregation. Preventing Aβ aggregation can be accomplished using various compounds such as dopamine[34] and heparin[35]. All of these agents work through similar mechanisms, reducing cytotoxicity by inhibiting and delaying aggregation of Aβ[36]. A non-reducing disaccharide with an α (1-1) linkage, trehalose, is the focus of much attention in view of its inhibitory effect on protein aggregation[37]. It has been reported that trehalose inhibits Huntington's disease *in vivo*[38] and amyloid formation of proteins (insulin[39] and Aβs[40]) *in vitro* owing to the hydration of proteins by the water-like hydrogen bond. Because the inhibitory effect of trehalose is not sufficient for medicinal application, an ingenious modification is required to amplify its activity. The biological abilities of saccharides can be amplified by multivalency[40]. Many groups have reported the multivalent effect and the generation of the multivalent compounds such as glycopeptides[41], glycocalixarenes [42], glycodendrimers[43] and glycopolymers[44]. In particular, glycopolymers carrying a saccharide at the side chain exhibit a large multivalent effect and applicability as biomaterials. Also they have studied various glycopolymers with biorecognition abilities, such as lectin recognition[45]; hepatocyte culture; and amyloid inhibitor[46].

EXPERIMENTAL

Plant materials and treatments: Tobacco (*Nicotiana tobacum* L.C.V E1) plants were grown in pots containing vermiculite at 25 ºC with continuous light. One-month seedlings were used in the following treatments. Five leaves were prepared per sample and three biological replicates were prepared per time point.

Nicotiana tobacum tissue was (10 to 100 mg fresh weight) disrupted in a mortar and pestle under liquid nitrogen. Ground tissue was added to pre-weight tube containing 1 mL methanol and 50 µL ribitol internal standard (1.3 mM), reweighed vortexed briefly and then extracted at 70 ºC for 15 min with shaking-samples were subsequently centrifuged at 20,000 g for 3 min. The methanol phase was reserved and the pellet

re-extracted with 500 µL H_2O and 375 µL chloroform for 5 min at 37 ºC (with shaking) and then centrifuged at 20,000 g for 3 min. The polar was recovered and added to the reserved methanol extract. The remaining organic phase was discarded. The qualitative test was carried out by GC/MS.

Gas chromatography and mass spectroscopy analysis

Method of analysis: A wet needle of the essential oil was inserted directly into the inlet (spitless mode) of a Hewlett Packard 6890 Gas chromatograph. The temperature of the injection port was set at 220 ºC, while the pressure at the inlet was maintained at 3.96 psi. A HP-5MS (cross linked 5 % phenyl methyl siloxane) column (30 m × 0.25 mm × 0.25 um film thickness) was temperature programmed from 60 to 150 ºC at 3 ºC/min after a 3.5 min delay. Helium was used as a carrier gas at 0.7 mL/min. Mass spectra was recorded by a HP 5937 series mass selective detector (MSD)[47].

RESULTS AND DISCUSSION

Trehalose is a naturally occurring disaccharide with known protein and membrane stabilizing capability. Because of these unique chemical properties, this molecule has been the focus of study in several neurodegenerative diseases, which are associated with the misfolding of disease-specific proteins. These conditions include Alzheimer"s disease an amyloid proteinopathy, Huntington"s disease (HD), an expanded polyglutamine proteinopathy and oculopharyngeal muscular dystrophy (OPMD), an expanded polyalanine proteinopathy. The aggregation behaviour of Aβ(1-42) was investigated by adding trehalose additives. The addition of trehalose attenuated the aggregation properties of the peptide. When α-crystallin was incubated in the presence of trehalose, the larger aggregates nearly disappeared since about 95 % of the globular structures were lower than 3 nm. Trehalose benefit was first shown in Huntington's model systems[48].

In addition, trehalose ability to stabilize protein structure has been suggested to strongly affect the tetrahedral hydrogen bond network of water and strengthen intermolecular O-H interactions with a kosmotrope character[49-51]. Molecular dynamic simulation results[52] have shown that trehalose can influence water activity by the formation of long lived hydrogen bonds with water molecules. These observations could provide a possible explanation about the trehalose effects on α-crystallin aggregates. The propensity to progressively turn low-molecular weight α-crystallin into high-molecular weight aggregates can be increased as a result of environmental factors that cause destabilization; under such conditions, α-crystallin can unfold or partially unfold losing its structural properties and assembly into higher order aggregates.

Trehalose, as showed by differential scanning calorimetry measurements, stabilizes the folded state of the protein against thermal denaturation, therefore inhibiting the formation of high-molecular weight aggregates. Moreover, the kosmotrope character of trehalose could stabilize not only the protein native conformation, but also the protein against attractive protein-protein intermolecular forces making α-crystallin low-molecular weight aggregates less energetically favourable. The results presented here provide more insights on the ability of trehalose to inhibit protein aggregation. This disaccharide stabilizes the α-crystallin structure and reduces either the sizes of preformed α-crystallin low-molecular weight aggregates, not affecting its chaperone activity, or the sizes of high-molecular weight aggregates. Further ongoing experiments are devoted to the study of the molecular basis of the protective features of trehalose activity and its role in the α-crystallin changes. Supplementation with anti-oxidants and trehalose has been proven to maintain the viability and motility of liquid or cryopreserved sperm cells of several mammalian species, *e.g.* the ram[53], goat[54], bull[55] and turkey[56].

Also, trehalose has been shown to inhibit Arabidopsis seedling root elongation and cause starch accumulation in shoots. Furthermore, trehalose increases AGPase (ADP-Glc pyrophosphorylase) activity and induces APL3 gene expression[57,58]. In soybean, trehalose also affects Suc synthase and invertase activities[59]. However, trehalose affects plant gene expression, enzyme activities, photosynthetic activity and carbon allocation is not clear, but trehalose-6-phosphate does not appear to have any effect on plant hexose phosphorylation[60]. However, transgenic tobacco plants expressing *Escherichia coli* homologs of trehalose-phosphate synthase and trehalose-6-phosphate phosphatase show a positive correlation between trehalose-6-phosphate levels and photosynthetic activity, suggesting a regulatory role for trehalose-6-phosphate in plant carbohydrate metabolism[61].

In this study, *Nicotiana tobacum*, growthing was analyzed leaves of *Nicotiana tobacum* for the presence of trehalose. Using as anion-exchange gas chromatography-mass spectrometry analysis, trehalose was in leaves (Fig. 1).

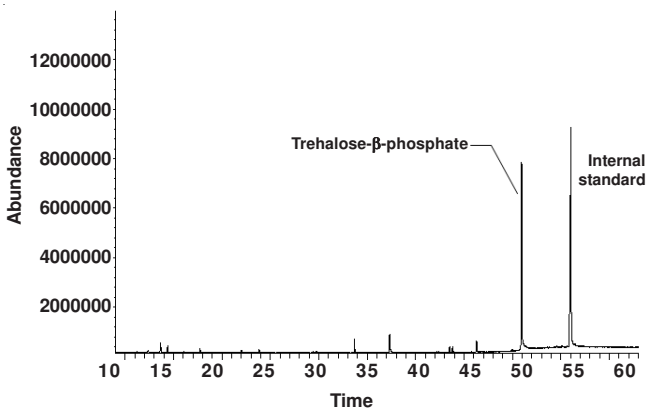


Fig. 1.

The carbohydrate was extracted from tobacco tissue and derivatized according to the procedures as reported above. The retention time of the commercial trehalose was same as that of the sample with retention time of 50.11 min (Fig. 1). Despite the extreme complexity of the plant chromatogram, this peak was unambigously identified as trehalose by comparison with the trehalose mass spectrum.

The distribution and activity of trehalase was measured in mature *Nicotiana tobacum*. In these plants, a strong trehalase activity was found in mature leaves, had significantly up activities (Fig. 1). Accordingly, its structure/activity benefits would be expected to persist for relatively extended times. If planned, studies demonstrate brain or cerebral spinal fluid absorption, trehalose will open a new avenue of potential

therapy for the prevention and treatment of multiple neurodegenerative diseases. This review summarized evidence for protective benefit in models of Huntingtin's disease, oculopharyngeal muscular dystrophy, Alzheimer's and α-crystallin. In Iran, 8000 acres of land is covered by tobacco. This plant is used to make cigarette which isn't good for human being and sometimes used in research but, it has a lot of attribute for diseases and we can analyze essential oil from it and make drug in place of cigarette. Although not studied, Parkinson's disease and amyotrophic lateral sclerosis display aggregate pathology that may be amenable to similar response.

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REFERENCES

1. A. Becker, P. Schloder, J.E. Steele and G. Wegener, *Experientia,* **52,** 433 (1996).
2. J.H. Crowe, J.F. Carpenter and L.M. Crowe, *Ann. Rev. Physiol.*, **60**, 73 (1998).
3. J.H. Crowe, F.A. Hoekstra and L.M. Crowe, *Ann. Rev. Physiol*., **54**, 579 (1992).
4. A.D. Elbein, *Adv. Carbohyd. Chem. Biochem.*, **30**, 227 (1974).
5. H.M. Giaever, O.B. Styryold, I.A. Kassen and R. Strom, *J. Bacteriol.*, **170**, 2841 (1988).
6. A. Vandercammen, J. Francois and H.G. Hers, *Eur. J. Biochem.*, **182**, 613 (1989).
7. A. Wiemken, *Antonie van Leeuwenhoek*, **58**, 209 (1990).
8. M.F. Wolschek and C.P. Kubicek, *J. Biol. Chem.*, **272**, 2729 (1997).
9. J.H. Crowe and L.M. Crowe and D. Chapmann, *Science,* **223**, 73 (1984).
10. J. Muller, A. Wiemken and R. Aeschbacher, *Plant Sci*., **147**, 37 (1999).
11. A.D. Elbein, Y.T. Pan, I. Pastuszak and D. Carroll, *Glycobiology*, **13**, 17 (2003).`
12. S. Fillinger, M.K. Chaveroche, P. van Dijck, R. de Vries, G. Ruijter, J. Thevelein and C. d'Enfert, *Microbiology,* **147**, 1851 (2001).
13. A.K. Gary, J.K. Kin, T.G. Owens, A.P. Ranwala, Y.D. Choi, L.V. Kochian, R.J. Wu, *Proc. Nat. Acad Sci. USA*, **99**, 15898 (2002).
14. J.H. Crowe, F.A. Hoekstra and L.M. Crowe, *Ann. Rev. Plant Physiol.*, **54**, 579 (1992).
15. R.A. Aeschbacher, J. Muller, T. Boller and A. Wiemken, *Plant Physiol.*, **119**, 489 (1999).
16. J. Muller, R.A. Aeschbacher, A. Wingler, T. Boller and A. Wiemken, *Plant Physiol*., **125**, 1086 (2001).
17. S. Rezvani and S. Shariati, *Am. J. Plant Physiol.*, **5**, 1 (2010).
18. O. Goddijn and S. Smeekens, *Plant J.*, **14**, 143 (1998).
19. N. Ren and M.P. Timko, *Genome,* **44**, 559 (2001).
20. S. Hasani, Pyrdshty, R. Mesbah and N. Babayyan, *Seed and Plant*, **24** 1387.
21. A.S. Qolizadeh and H. Far Ahi, *Tobacco Res. Inst .Persian month Tir Tash.,* pp. 21-45 (1382) (in Persian).
22. A. Qolizadeh and Gh. Mirzaei, 1384. Effects of Different Nitrogen Fertilizers in Irrigation Water Absorbing Chlorine in Virginia Tobacco (cultivar Cochrane 347). Research record. Tyrtash Tobacco Research Institute, pp. 145-159.
23. H. Bloemendal, Molecular and Cellular Biology of the Eye Lens, Wiley, New York, (1981).
24. T. Bando, S. Kosaka, C. Liu, T. Hirai, T. Hirata, H. Yokomise, K. Yagi, K. Inui, S. Hitomi and H. Wada, *Thorac. Cardiovasc. Surg.*, **108**, 92 (1994).
25. T. Neta, K. Takada and M. Hirasawa, *J. Dent.*, **28**, 571 (2000).
26. A. Lopez-Saaz, N. Ortiz, L. Gallego, J.J. Gadre, *Arch. Androl*., **44**, 155 (2000).
27. C. Yildiz, A. Kaya, M. Aksoy and T. Tekeli, *Theriogenology,* **54**, 579 (2000).
28. D.J. Selkoe, *Physiol. Rev*., **81**, 741 (2001).
29. D.J. Selkoe, *Ann. Rev. Nuerosci*., **17**, 489 (1994).
30. G.G. Glenner and C.W. Wong, *Biochem. Biophys. Res. Commun.*, **122** 1131 (1984).
31. G.G. Glenner and C.W. Wong, *Biochem. Biophys. Res. Commun*., **120** 885 (1984).
32. S. Sinha and I. Lieberbur, *Proc. Natl Acad. Sci. USA,* **96**, 11049 (1999).
33. L.K. Thompson, *Proc. Nat. Acad. Sci. USA,* **100**, 383 (2003).
34. C. Opazo, X. Huang, R.A. Cherny, R.D. Moirll, A.E. Roher, A.R. White, R. Cappai, C.L. Masters, R.E. Tanzill, N.C. InestrosaDagger and A.I. Bush, *J. Biol. Chem*., **277**, 40302 (2002).
35. Y. Yoshiike, K. Tanemura, O. Murayama, T. Akagi, M. Murayama, S. Sato, X. Sun, N. Tanaka and A. Takashima, *J. Biol. Chem*., **276**, 32293 (2001).
36. C. Janus, *CNS Drugs*, **17**, 457 (2003).
37. R. Liu, H. Barkhordarian, S. Emadi, C.B. Park and M.R. Sierks, *Neurobiol. Dis.*, **20**, 74 (2005).
38. M. Tanaka, Y. Machida, S. Niu, T. Ikeda, N.R. Jane, H. Doi, M. Kurosawa, M. Nekooki and N. Nukina, *Nat. Med*., **10**, 148 (2004).
39. A. Arora, C. Ha and C.-B. Park, *FEBS Lett*., **564**, 121 (2004).
40. M. Mammen, S.-K. Choi and G.M. Whitesides, *Angew. Chem. Int. Ed*., **37**, 2754 (1998).
41. J.J. Lundquist, S.D. Debenham and E.J. Toone, *J. Org. Chem*., **65**, 8245 (2000).
42. A. Dondroni, M. Kleban, X. Hu, A. Marra and H.D. Banks, *J. Org. Chem*., **67**, 4722 (2002).
43. R. Roy and J.M. Kim, *Angew. Chem. Int. Ed*., **38**, 369 (1999).
44. A. Kobayashi, T. Akaike, K. Kobayashi and H. Sumitomo, *Makromol. Chem. Rapid. Commun.*, **7**, 645 (1986).
45. Y. Miura, T. Ikeda and K. Kobayashi, *Biomacromolecules,* **4**, 410 (2003).
46. Y. Miura, Y. Yasuda, K. Yamamoto, M. Koike, Y. Nishida and K. Kobayashi, *Biomacromolecules,* **8**, 2129 (2007).
47. C. Joseph, Touchstone, Practice of Thin Layer Chromatography (1992).
48. M. Tanaka, Y. Machida, S. Niu, T. Ikeda, N.R. Jana, H. Doi, M. Kurosawa, M. Nekooki and N. Nukina, *Nat. Med.*, **10**, 148 (2004).
49. A. Cesaro, V. Magazu, F. Migliardo, F. Sussich and M. Vadala, *Physica B*, **350** (1-3, Suppl. 1), e367 (2004).
50. A. Hedoux, J.F. Willart, R. Ionov, F. Affouard, Y. Guinet, L. Paccou, A. Lerbret, M.J. Descamps, *Phys. Chem. B,* **110**, 22886 (2006).
51. S. Magazu, F. Migliardo and A.J. Ramirez-Cuesta, *Int. J. Phys. Sci*., **1**, 75 (2006).
52. Y. Choi, K.W. Cho, K. Jeong and S. Jung, *Carbohyd. Res*., **341**, 1020 (2006).
53. W.M.C. Maxwell and T. Stojanov, *Reprod. Fertil. Dev.*, **8**, 1013 (1996).
54. I. Salvador, J. Yaniz, M.P. Viudes-de-Castro, E.A. Gomez and M.A. Silvestre, *Theriogenology*, **66**, 974 (2006).
55. R.H. Foote, C.C. Broket and M.T. Kaproth, *Anim. Reprod. Sci*., **71**, 13 (2002).
56. A.M. Donoghue and D.J. Donoghue, *Poult. Sci*., **76**, 1440 (1997).
57. T. Fritzius, R. Aeschbacher, A. Wiemken and A. Wingler, *Plant Physiol*., **126**, 883 (2001).
58. A. Wingler, T. Fritzius, A. Wiemken, T. Boller and R. Aeschbacher, *Plant Physiol*., **124**, 105 (2000).
59. J. Müller, T. Boller and A. Wiemken, *J. Plant Physiol.*, **153**, 255 (1998).
60. A. Wiese, F. Groner, U. Sonnewald, H. Deppner, J. Lerchl, U. Hebbeker, U. Flugge and A. Weber, *FEBS Lett*., **461**, 13 (1999).
61. M. Paul, T. Pellny and O. Goddijn, *Trends Plant Sci*., **6**, 197 (2001).