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Insight into the role of catalases in salt stress in potato (*Solanum tuberosum* L.)

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In order to investigate a possible link between catalase (CAT) activity and salinity tolerance, an *in vitro* and *in vivo* study of the behavior of transgenic lines of potato (cv. 'Désirée') under salt stress conditions was carried out. Three groups of transgenic lines and non transformed control (DWT) were used in this study: lines expressing a bacterial catalase gene and lines repressing catalase activity by either co-suppression or anti-sense strategies. Various concentrations of NaCl were tested: *in vitro* 0, 25, 50 and 75 mM and *in vivo* 25, 50 and 75 mM. The results of this work show that the genetic modification of CAT activity affects the multiplication rate of vitroplants, as well as vegetative and physiological growth parameters under salt stress conditions. At 25, 50 and 75 mM of NaCl, over-expression (line KatE16) and repression of CAT increased and reduced respectively the multiplication rate of vitroplants. Differences between the transgenic lines and the wild type were evident in tuber yield and leaf chlorophyll content. These parameters were significantly increased in CAT over-expressing and slightly decreased in SU3 line repressed in CAT under 25 mM of salt stress. A stability of the potential quantum yield (Fv/Fm) was observed in the lines over-expressing the CAT at 25, 50 and 75 mM of NaCl. The repression of CAT was associated with a decrease of Fv/Fm value at 50 mM of NaCl. These results show that catalases contribute to salinity tolerance mechanisms in potato.
Keywords. Catalase, NaCl, *Solanum tuberosum* L.

Aperçu sur le rôle des catalases dans le stress salin chez la pomme de terre (*Solanum tuberosum* L.). Dans le but d'étudier d'éventuelles relations entre l'activité catalase (CAT) et la tolérance à la salinité, une étude de comportement *in vitro* et *in vivo* de lignées transgéniques de pomme de terre (cv. 'Désirée') sous conditions de stress salin a été effectuée. Trois groupes de lignées transgéniques et un témoin non transformé (DWT) ont été utilisés dans cette étude : des lignées sur-exprimant une catalase bactérienne et des lignées réprimées en activité CAT par des stratégies anti-sens ou de co-suppression. Différentes concentrations de NaCl ont été testées : *in vitro* 0, 25, 50 et 75 mM et *in vivo* 25, 50 et 75 mM. Les résultats de ce travail montrent que la modification génétique de l'activité CAT affecte le taux de multiplication des vitroplants, ainsi que des paramètres physiologiques et de croissance sous conditions de stress salin. À des concentrations de 25, 50 et 75 mM de NaCl, la sur-expression (lignée KatE16) augmente le taux de multiplication des vitroplants et la répression le réduit. Des différences entre les lignées transgéniques et le type sauvage (non transformé) dans le rendement en tubercules et le contenu en chlorophylle des feuilles ont été observées. Ces paramètres ont augmenté significativement chez les lignées sur-exprimant la CAT et ont légèrement diminué chez la lignée SU3 partiellement réprimée en CAT sous 25 mM de stress salin. Une stabilité dans le rendement quantique (Fv/Fm) a été observée chez les lignées sur-exprimant la CAT aux concentrations de 25, 50 et 75 mM de NaCl. La répression de la CAT a été associée à une diminution de la valeur Fv/Fm au niveau 50 mM de NaCl. Ces résultats montrent que la CAT contribue aux mécanismes de tolérance à la salinité chez la pomme de terre.
Mots-clés. Catalase, NaCl, *Solanum tuberosum* L.

1. INTRODUCTION

Water salinity is a complex and harmful threat faced by plants; due to disruption of ionic, osmotic, and cell-water homeostasis (Munns, 1993). At the cellular level, salinity in general and NaCl in particular cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic

dysfunction, including photosynthesis (Fridovich, 1986; Imlay et al., 1988). Molecular and biochemical studies of salt stress responses in plants demonstrated significant increases of active oxygen species (AOS), including singulet oxygen ($^1O_2$), superoxyde anion (O_2^-) and hydrogen peroxide (H_2O_2) (Jugklang et al., 2004; Tsai et al., 2004). To neutralize and repair the damage initiated by AOS, plants have developed a complex antioxidant system: superoxyde dismutase (SOD) neutralizes the O_2^- and produces H_2O_2, while H_2O_2 neutralization uses other enzymes such as catalase (CAT) and ascorbate peroxidase (APX). A direct connection between antioxidant defense systems and environmental stresses, including salinity was reported in many researches. Thermal stress was associated with more activation of CAT activity in adapted clones of gladiolus (Bettaieb et al., 2007) and transgenic rice (Matsumura et al., 2002) as compared to their controls. Water stress was associated with an increase of the antioxidant enzyme response and induction of new CAT isoforms in rice (Srivalli et al., 2003). Salt-tolerant cotton cultivars exhibited significantly greater in CAT, APX and SOD activities as compared to the salt-sensitive ones (Gossett et al., 1994). In a related study, *in vitro* selection for salt stress-resistance showed a significant increase in antioxidant enzyme activities of cotton cell lines grown under NaCl stress (Gossett et al., 1996). Modulation by NaCl of SOD, CAT and APX activities was studied in *Nicotiana plumbaginifolia* (Savouré et al., 1999). In rice, Benavente et al. (2004) have reported an alteration of the gene expression encoding for antioxidant enzyme under salt stress. Although results of these investigations show the role of antioxidant status in the adaptation of many plants species to salt stress conditions, little information is nowadays available on potato. This work was carried out in order to establish an eventual relationship between CAT activity and salinity tolerance in this species. Transgenic lines (modified in their CAT activity) were used for an *in vitro* and *in vivo* study of their behavior under salt stress conditions.

2. MATERIALS AND METHODS

2.1. Biological material

Three groups of transgenic lines and a non transformed control (DWT) of potato cv. 'Désirée' were used in this work. The transgenic lines were generated using different strategies. Genes coding for CAT2 of *Nicotiana plumbaginifolia* (*Cat2*, antisense) and of *Gossypium hirsutum* (*SU2*, sense) were used for the partial repression of CAT activity (2AS lines and SU lines, respectively). The *KatE* gene coding for *Escherichia coli* (strain DH5α) HPII CAT was used to generate lines over-expressing CAT activity in plastids. Two constructs pCat2AS (*Cat2*, anti-sense) and pCatGH (*SU2*, sense) were kindly provided by Prof. D. Inze (Gent University, Belgium) (see Chamnongpol et al., 1996 for more details), *KatE* was cloned in a binary vector pBin19 (kindly provided by Prof. U. Sonnewald, Institute of Plant Genetics and Crop Plant Research, Germany) (Bajji et al., 2007). The different constructs were mobilized into *Agrobacterium tumefaciens* (strain 58 for pCat2AS and pCatGH and strain LBA4404 for pBin19) to transform potato internodal explants as described by Beaujean et al. (1998) and modified by M'Hamdi et al. (2003).

2.2. Salt treatments

The behavior study under salt stress conditions of the transgenic lines modified in their CAT activity was carried out under *in vitro* and *in vivo* conditions. Four concentrations of NaCl (0, 25, 50 and 75 mM) were added in the *in vitro* Murashige and Skoog culture medium. *In vivo*, under semi-controlled greenhouses conditions, the plants were irrigated with water containing one of three NaCl concentrations (25, 50 and 75 mM) throughout the growth cycle. Concentration of 25 mM NaCl is the mean value of normal water irrigation in our conditions and it was used as a control in this study.

2.3. Biochemical characterization of transgenic lines

The Laemmli method (1970) but without sodium dodecyl sulfate (SDS) was used for characterization of CAT activity in the transgenic lines. Soluble protein samples (50 µg per sample) were subjected to non-denaturing 10% PAGE. CAT isozymes were detected on the gel as follows: the gel was washed 3 times (15 min each) with distilled water, then incubated for 10 min in 0.88 mM H_2O_2 solution, rinsed again with distilled water, and finally incubated with 1% w/v of ferric chloride and potassium ferricyanide solution until bands appeared (yellow bands on a green background).

2.4. Measured parameters

Vegetative growth and tuber yield. In the *in vitro* tests, our observations were made on the number of internodes three weeks after cultivation. In the *in vivo* tests, the final tuber yield and physiological parameters (chlorophyll fluorescence and content) were measured. In this work which was carried out for three generations both *in vivo* and *in vitro*, each observation was made on 20 plants and repeated three times.

Chlorophyll fluorescence. A rotary system type FIM 1500 Analytical Development Limited, ADC was used to measure chlorophyll fluorescence from 90-day-old mature leaves.

Chlorophyll content. Chlorophyll was extracted by homogenizing and boiling 1 g of fresh weight leaves in 35 ml ethanol 96%. After centrifugation for 10 min at 4,000 *g*, the chlorophyll content was determined spectro-photometrically from the ethanolic supernatant at 654 nm, as described by Wintermans et al. (1965).

2.5. Statistical analysis

All data obtained were subjected to analysis of variance at 5% level using SAS program.

3. RESULTS

3.1. Biochemical characterization of transgenic lines

The characterization of the transgenic lines showed a significant reduction in one of the CAT isoforms in the 2AS and SU transgenic lines groups as compared to the control (**Figure 1**). The arrow of **figure 1** indicates the repressed band in some transgenic lines. Data derived from densitometric analysis showed a decrease of up to 66% of CAT activity in these lines. In the case of KatE lines, both spectrophotometric and native gel analyses showed that their CAT activity was significantly increased, between 70 and 147% in percoll-enriched chloroplast fraction in comparison with the wild type (**Figure 2**). Among all transgenic lines transformed and PCR positive, 20% showed modifications in their CAT activity.

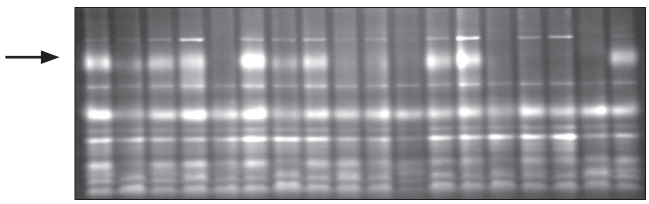

Figure 1. Native-PAGE showing catalase activity of two transgenic lines groups: SU transformed for repressing catalase activity by co-suppression (from SU1 to SU12) and 2AS transformed for repressing catalase activity by anti-sense (from 2AS62 to 2AS65) compared to the non transformed control cv. 'Désirée' Wild Type (DWT) — *Gel non dénaturant montrant l'activité catalase de deux groupes de lignées transgéniques : SU réprimées par co-suppression (de SU1 à SU12) et par anti-sens (de 2AS62 à 2AS65) comparées au témoin cv. 'Désirée' non transformé (DWT).*

The arrow indicates the position of the repressed band in some transgenic lines (SU4, SU8, SU9, SU10, 2AS62, 2AS63, 2AS64 and 2AS65) — *La flèche indique la position de la bande réprimée chez certaines lignées (SU4, SU8, SU9, SU10, 2AS62, 2AS63, 2AS64 et 2AS65)*; Transgenic lines SU3, SU5, SU11 and SU12 over-expressed catalase activity. SU1, SU2 and SU7 did not show clear catalase activity repressions. These lines were not used further in this study — *Les lignées SU3, SU5 SU11 et SU12 sur-expriment l'activité CAT. SU1, SU2 et SU7 ne montrent pas de répression claire. Ces lignées n'ont pas été utilisées dans cette étude.*

3.2. Vegetative growth and tuber yield

For *in vitro* experiments, the vegetative growth was estimated using the number of internodes per vitroplant of three week-old culture. This parameter measuring the multiplication rate presented some variations according to the modification sense of CAT activity. A higher number of internodes per vitroplant was observed in line16 over-expressing CAT activity as compared to the control at 25, 50 and 75 mM of NaCl (**Figure 3**). On the contrary, the repression of the CAT activity by "anti-sense" or "co-suppression" was associated with a reduction of the multiplication rate

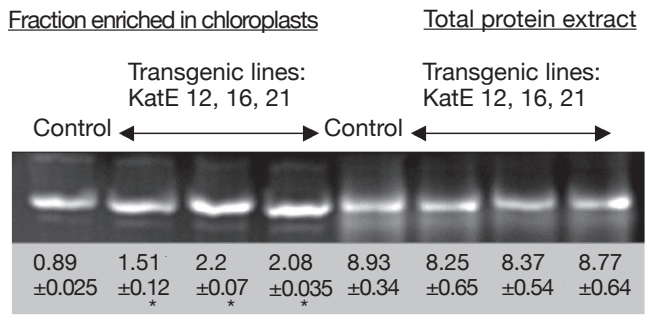

Figure 2. Native-PAGE showing catalase activity of transgenic lines over-expressing the bacterial catalase KatE compared to the non transformed control cv. 'Désirée' Wild Type — *Gel non dénaturant montrant l'activité catalase de lignées transgéniques sur-exprimant la catalase bactérienne KatE comparée au témoin non transformé cv. 'Désirée'.*

The values indicate CAT activity (µmole H_2O_2 per min per mg of proteins) — *Les valeurs indiquent l'activité CAT (µmole H_2O_2 par min par mg de protéines);* Each value represents mean ± standard error — *Chaque valeur représente la moyenne ± l'erreur standard*; the asterisk (*) indicates significant difference between a transgenic line and the wild type at the 5% level — *Le symbole (*) indique des différences significatives entre les lignées transgéniques et le témoin au niveau* $\alpha = 5$ %; Differences and similarities between transgenic lines and the control in CAT activity respectively in the fraction enriched in the chloroplasts and total protein extract confirmed the ectopic expression of bacterial catalase — *Des différences et des similitudes dans l'activité CAT entre les lignées transgéniques et le témoin respectivement au niveau de la fraction enrichie en chloroplastes et l'extrait protéique total confirme l'expression ectopique de la catalase bactérienne.*

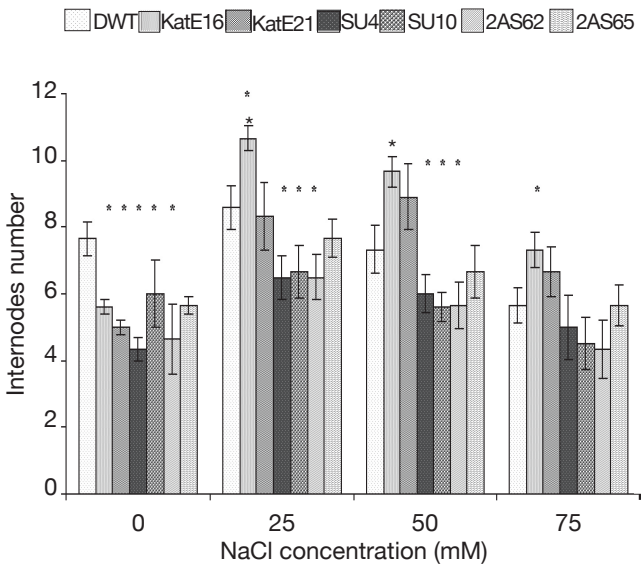

Figure 3. Effect of three NaCl concentrations added to the culture medium on the number of internodes per vitroplant after three weeks of subculture of three transgenic lines groups (KatE: lines over-expressing catalase activity, SU and 2AS: lines repressing catalase activity respectively by co-suppression and anti-sense) compared to the non-transformed control (DWT) — *Effet de trois doses de NaCl additionnées au milieu de culture sur le nombre d'entre-nœuds par vitroplant après trois semaines de subculture chez trois groupes de lignées transgéniques (KatE : lignées sur-exprimant l'activité catalase, SU et 2AS : lignées réprimées dans l'activité catalase respectivement par co-suppression et anti-sens) comparées au témoin non transformé (DWT).*

Each value represents mean ± standard error — *Chaque valeur représente la moyenne ± l'erreur standard*; The asterisk (*) above histograms indicates significant differences between a transgenic line and the wild type at the 5% level — *Le symbole (*) au-dessus des barres indique des différences significatives entre les lignées transgéniques et le témoin au niveau* $\alpha = 5\ \%$.

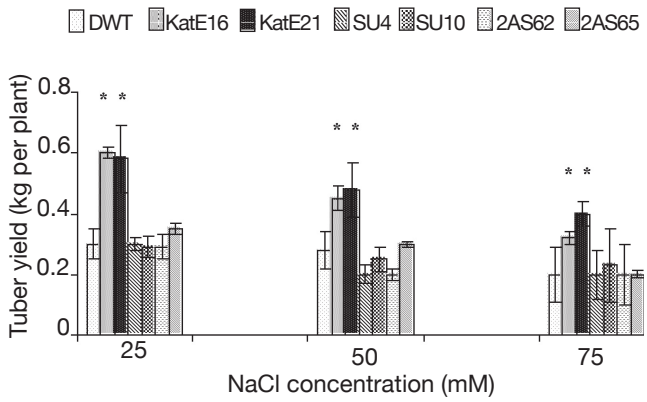

Figure 4. Effect of three concentrations of NaCl added to the irrigation water on the tuber yield (kg per plant) of three transgenic lines groups (KatE: lines over-expressing the catalase activity, SU and Cat2AS: lines repressing the catalase activity respectively by co-suppression and anti-sense) compared to the non-transformed control (DWT) — *Effet de trois doses de NaCl additionnées à l'eau d'irrigation sur le rendement en tubercules (kg par plante) chez trois groupes de lignées transgéniques (KatE : lignées sur-exprimant l'activité catalase, SU et 2AS : lignées réprimées dans l'activité catalase respectivement par co-suppression et anti-sens) comparées au témoin non transformé (DWT).*

Each value represents mean ± standard error — *Chaque valeur représente la moyenne ± l'erreur standard*; The asterisk (*) above histograms indicates significant differences between a transgenic line and the wild type at the 5% level — *Le symbole (*) au-dessus des barres indique des différences significatives entre les lignées transgéniques et le témoin au niveau* $\alpha = 5\ \%$.

compared to that of the control under 25 and 50 mM of NaCl (**Figure 3**). The highest number of internodes per vitroplant in both the transgenic and the control lines was observed at 25 mM NaCl. The tuber yield is an important agronomical parameter and was affected by CAT modification in this study. Significant differences were observed between the lines over-expressing CAT activity and the wild type. These lines gave the highest tuber yield in all used NaCl concentrations (**Figure 4**). In the contrary, repressed lines did not show any differences with the control under the same conditions (**Figure 4**).

3.3. Physiological parameters

The potential quantum yield (Fv/Fm), which is an indicator of the photosystemII (PSII) efficiency was affected under salt stress depending on the CAT modification (**Figure 5**). Q stability and a reduction of Fv/Fm values with increasing NaCl concentrations were measured respectively in the lines that over-express and repress CAT. The lowest values for all NaCl concentrations used were measured in SU4 line repressing CAT activity by "co-suppression" and which gave the lowest tubers yield, FM. The lines over-expressing CAT activity gave Fv/Fm values near to 0.8 and were comparable with those measured under non stressing conditions. In these lines, the tuber yield was higher (**Figure 4**). A non-transformed control had an intermediate situation between the two transgenic lines categories for the potential quantum yield.

The transgenic lines that over-express CAT activity showed superiority in chlorophyll content and gave the highest, Fv/Fm and the tuber yield. CAT repression was associated to a reduction of the pigment content only in SU4 line at 25 mM of NaCl (**Figure 6**).

4. DISCUSSION

The internodes number and the tuber yield measured were affected by CAT modification under salt stress conditions. The lines over-expressing CAT activity

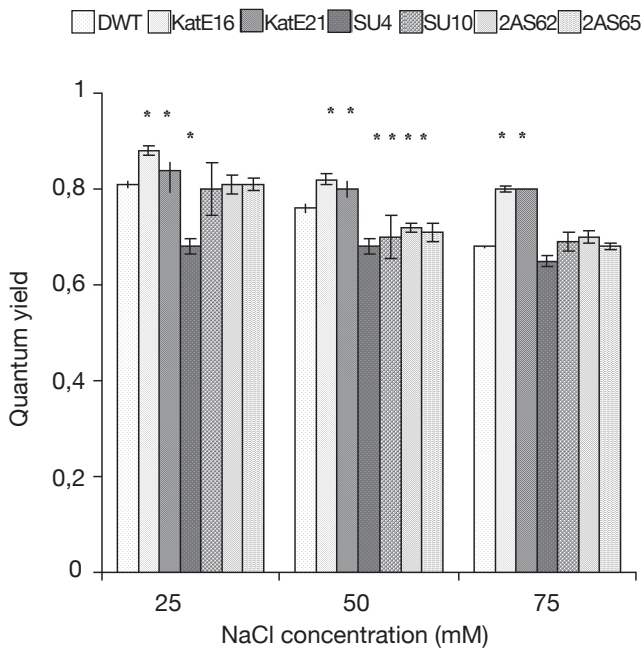

Figure 5. Effect of three NaCl concentrations added to the irrigation water on the potential quantum yield (Fv/Fm) of three transgenic lines groups (KatE: lines over-expressing catalase activity, SU and 2AS: lines repressing catalase activity respectively by co-suppression and anti-sense) compared to the non-transformed control (DWT) — *Effet de trois doses de NaCl additionnées à l'eau d'irrigation sur le rendement quantique (Fv/Fm) chez trois groupes de lignées transgéniques (KatE : lignées sur-exprimant l'activité catalase, SU et 2AS : lignées réprimées dans l'activité catalase respectivement par co-suppression et anti-sens) comparées au témoin non transformé (DWT).*

Each value represents mean ± standard error — *Chaque valeur représente la moyenne ± l'erreur standard*; The asterisk (*) above histograms indicates significant differences between a transgenic line and the wild type at the 5% level — *Le symbole (*) au-dessus des barres indique des différences significatives entre les lignées transgéniques et le témoin au niveau* $\alpha = 5\ \%$.

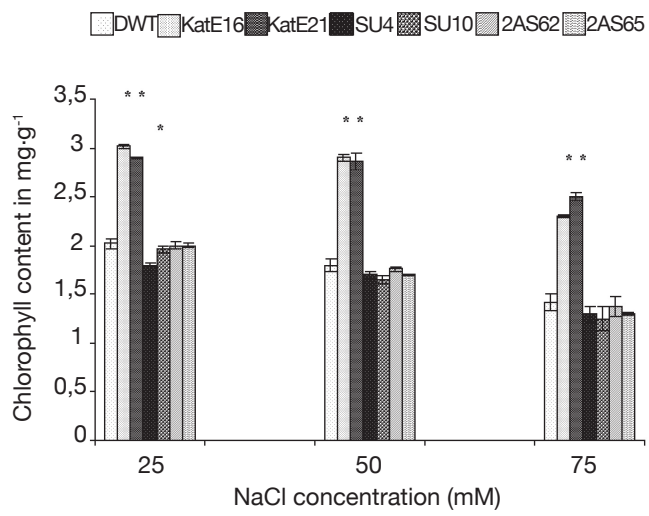

Figure 6. Effect of three NaCl concentrations added to the irrigation water on the chlorophyll content ($mg \cdot g^{-1}$ of MF) of three transgenic lines groups (KatE: lines over-expressing catalase activity, SU and Cat2AS: lines repressing catalase activity respectively by co-suppression and anti-sense) compared to the non transformed control (DWT) — *Effet de trois doses de NaCl additionnées à l'eau d'irrigation sur le contenu en chlorophylle ($mg \cdot g^{-1}$ de MF) chez trois groupes de lignées transgéniques (KatE : lignées sur-exprimant l'activité catalase, SU et 2AS : lignées réprimées dans l'activité catalase respectivement par co-suppression et anti-sens) comparées au témoin non transformé (DWT).*

Each value represents mean ± standard error — *Chaque valeur représente la moyenne ± l'erreur standard*; The asterisk (*) above histograms indicates significant differences between a transgenic line and the wild type at the 5% level — *Le symbole (*) au-dessus des barres indique des différences significatives entre les lignées transgéniques et le témoin au niveau* $\alpha = 5\ \%$.

gave the highest values for these parameters as compared to the control at 25, 50 and 75 mM of NaCl concentrations (**Figures 3** and **4**). The repression of CAT activity was associated with a decrease of the internodes number at 25 and 50 mM of NaCl and any change in tuber yield (**Figures 3** and **4**). In fact, isoform 2 of CAT is expressed in the stems (Willekens et al., 1995) but *KatE* was targeted in photosynthetic tissues (chloroplasts).

In several plant species, a relationship between AOS/antioxidant enzymes and the tolerance to abiotic stress was established: Sairam et al. (2002) observed in wheat an increase of H_2O_2 content, SOD, CAT and glutathione reductase (GR) activities under salt stress conditions. With barley lines repressed in 90% of CAT activity, Smith et al. (1984) measured a decrease of growth and noted necrosis development in light stressed leaves. In potato, Benavides et al. (2000) observed an increase in the reduced form of glutathion (GSH) in salinity tolerant clones compared to the sensitive ones. Glutathion is involved in the detoxication of H_2O_2. Willekens et al. (1997) reported that the CAT activity belongs to the normal operation of the photosynthetic apparatus in tobacco plants and is essential for the antioxidant defense in plant cells under stress conditions. They also measured a reduction of the photosynthetic activity estimated to more than 50% in the old leaves with reduced CAT activity. Matsumura et al. (2002) suggest that the improvement of low temperature tolerance in transgenic rice is associated with the increase in their capacity of H_2O_2 neutralization. Shikanai et al. (1998) observed an improvement of light tolerance, paraquat and drought stress in tobacco plants transformed with the same molecular construction as used in this work (*KatE* gene coding for HPII catalase of *E. coli* expressed in chloroplasts).

As far as physiological parameters are concerned, our results converge with those of Broetto et al. (2007) who indicate a significant reduction in the potential quantum yield in *Mesembryanthemum* sp. under salt and light stress conditions. On the other hand, Fedina et al. (2006) reported only a slight reduction of the chlorophyll fluorescence in barley under salt stress conditions but measured a strong increase in the H_2O_2 content. The reduction in chlorophyll content is a general phenomenon in salt-sensitive plants growing under salt stress conditions (Srivastava et al., 1988). In potato, Benavides et al. (2000) showed a reduction of the photosynthetic assimilation associated to a reduction of chlorophyll content and measured a 23% decrease in chlorophyll content in salt-sensitive clones. Our data for this parameter are in accordance with others: the over-expression of CAT activity was associated with high chlorophyll content and with the repression a decrease in this pigment was observed in SU4 line at 25 mM of NaCl.

All these results show that CAT activity is involved in the salinity mechanisms tolerance in potato and its usefulness as a biochemical marker for salt stress tolerance in breeding programs is worth investigating.

Acknowledgements

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