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Review

Review of recent transgenic studies on abiotic stress tolerance and future molecular breeding in potato

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Global warming has become a major issue within the last decade. Traditional breeding programs for potato have focused on increasing productivity and quality and disease resistance, thus, modern cultivars have limited tolerance of abiotic stresses. The introgression of abiotic stress tolerance into modern cultivars is essential work for the future. Recently, many studies have investigated abiotic stress using transgenic techniques. This manuscript focuses on the study of abiotic stress, in particular drought, salinity and low temperature, during this century. Dividing studies into these three stress categories for this review was difficult. Thus, based on the study title and the transgene property, transgenic studies were classified into five categories in this review; oxidative scavengers, transcriptional factors, and above three abiotic categories. The review focuses on studies that investigate confer of stress tolerance and the identification of responsible factors, including wild relatives. From a practical application perspective, further evaluation of transgenic potato with abiotic stress tolerance is required. Although potato plants, including wild species, have a large potential for abiotic stress tolerance, exploration of the factors responsible for conferring this tolerance is still developing. Molecular breeding, including genetic engineering and conventional breeding using DNA markers, is expected to develop in the future.

Key Words: potato (*Solanum tuberosum* L.), drought, salinity, low temperature, gene engineering technique.

Introduction

Since the 19th century, the CO_2 concentration in the atmosphere has been gradually increasing and recently reached 400 ppm at some observation locations (Japan Meteorological Agency 2014). Global warming has become a major issue in the last decade. Although it may be difficult to associate raising CO_2 concentrations with extreme weather conditions, such as heavy rain, drought, or low (high) temperatures, these conditions appear to be occurring with increased frequency (Japan Meteorological Agency 2013). Agriculture was established by adapting plants to grow in certain climatic conditions, thus, productivity depends on the weather conditions and typically decreases during, or following, periods of extreme weather (Boyer 1982). In addition, various human activities have resulted in saline or drought conditions, thereby decreasing the available area of arable land. There are reports that over 800 million ha of land throughout the world are saline, covering over 6% of the total land area (Munns 2005). Further, over 20% of arable land has salinity damage (Munns 2005). Irrigation has contributed to the availability of arable land, with recent estimates suggesting that around 20% of the world's arable land is irrigated and provides 40% of food and feed (Kondrak *et al*. 2012). However, 50% of irrigated land suffers from salinity damage (Geilfus *et al*. 2010). Some studies predict that 30% of arable land will be lost within 25 years and around half of arable land will be unavailable by 2050 (Wang *et al*. 2003). Currently, 70% of the world's fresh water is consumed by agriculture (Kondrak *et al*. 2012). Thus, effective utilisation of limited water resources is an important aspect to consider in the near future.

Potato is the fourth highest produced crop in the world and the most important non-cereal food crop (Pino *et al*. 2007), with world production estimated to be over 350 million tons in 2013 (http://faostat.fao.org). Potato is a versatile crop, and is utilized as not only food and feed but also in industrial materials and fuels (Ortiz and Watanabe 2004). Potato is a temperate or continental zone crop and is cultivated worldwide in areas such as Northern China, Europe, North America and the highlands of South America (Pino *et al*. 2007). Although detailed descriptions about the origin and history of the potato will not be discussed here, there are various types of wild relative species present in its original region of origin and their habitat ranges from cool highland zones to tropical lowlands (Hawkes 1994). The genetic

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variation of these wild relatives is expected to be high. Potato has limited abiotic stress tolerance (Shin *et al*. 2011, Vasquez-Robinet *et al*. 2008). In particular, drought stress is inimical for potato due to their shallow root system (Bouaziz *et al*. 2012), which has a rooting zone between 50 cm to 80 cm depth (Dalla Costa *et al.* 1997, Ekanayaka and Midmore 1992). In addition to drought stress, potato is vulnerable to salt stress. In an experimental evaluation, production decreased by half under 50 mM NaCl conditions, and was negligible under 150 mM NaCl conditions (Sherraf *et al*. 1994). In a field evaluation, production of potato was also reduced by half under 5.9 mS/m electric conductivity (EC) (Kotuby-amacher *et al*. 2000). Furthermore, potato suffers damage at –3°C and has no ability to acclimate to cold conditions (Chen and Li 1980). Therefore, of the temperate crops, potato is susceptible to low temperatures. From an agricultural point of view, frost is one of the most harmful low temperature conditions that causes damage to crops (Barrientos *et al*. 1994, Chen and Li 1980, Costa and Li 1993, Vega and Bamberg 1995).

Traditional breeding of crops has focused on increasing productivity and quality and disease resistance, thus, modern cultivars have limited abiotic stress tolerance. The cold tolerant wild species *Solanum commersonii* was used as a genetic donor of cold tolerance and cold acclimation for *S. tuberosum*. However, this breeding effort failed to confer cold tolerance traits to *S. tuberosum* (Cardi *et al*. 1993, Estrada 1982, Estrada *et al*. 1993, Iovene *et al*. 2004, Pavek and Corsini 2001). Potato is the one of the highest crops in the Harvest Index (HI), like sweet potato, and its value is over 60% (Mazurczyk *et al*. 2009). Since HI indicates the available portion of each crop, high HI crops are important for the establishment of sustainable societies in the future. Investigation of abiotic stress tolerance in potato is essential for future cultivation efforts and molecular breeding of potato, including genetic engineering, is expected to develop in anticipation of future crop requirements.

Recently, many studies have been undertaken to investigate abiotic stress in potato using transgenic techniques. This manuscript focuses on the three types of abiotic stress, drought, salinity and low temperature, and discusses recent studies from 2000 onwards. These three abiotic stresses have specific individual characteristics, but they also overlap, and it was difficult to clearly divide them into three categories. Furthermore, these environmental stresses are known to enhance the accumulation of ROS in plant cells. Various types of ROS result in the oxidation of many cellar components and lead to oxidative destruction of the cell (Asada and Takahashi 1987, Mittler 2002). Enhancement of the ROS scavenging system is expected to confer tolerance to various types of stresses in plants. On the other hand, transcriptional factors (TFs) govern various related downstream genes. In fact, overexpression of abiotic stress related TF also confers the abiotic stress tolerance to plants. Thus, in this review, based on the study title and the transgene property, transgenic studies were classified into five categories: oxidative scavengers, transcriptional factors and the three abiotic stresses, drought, salinity and low temperature (**Table 1**). In addition to transgenic potato, exploring studies of responsible genes for abiotic stress tolerance also are introduced in this review. Potato is high potential crop plant that can acquire abiotic stress tolerance by molecular breeding including conventional methods with molecular markers and GE techniques.

Drought

Under drought condition, cytoplasm is getting dehydrated and following reduction of various enzymatic activities. Furthermore, ions also accumulate along with pH change. This causes enzyme inactivation (Sinha 2004). Potato can grow under many different climatic conditions; however, its shallow root system makes it sensitive to drought (Pino *et al*. 2013) as it is thought to restrict water uptake (Dalla Costa *et al*. 1997, Ekanayake and Midmore 1992). Drought affects primary vegetative growth in potato, such as shoot length, leaf size and leaf number, and photosynthetic rate (Dalla Costa *et al*. 1997, Deblonde and Lendent 2001, Ekanayake and Midmore 1992, Kiziloglu *et al*. 2006, Weisz *et al*. 1994), and drought is a particularly harmful stress during tuber formation as it influences tuber production and quality (Deblonde and Lendent 2001, Ekanayake and Midmore 1992, Weisz *et al*. 1994). Thus, avoidance of drought stress is one of the most important issues in potato production.

One strategy used by plants to adapt to drought is the accumulation of compatible solutes. Glycine betaine (GB) is a general osmoprotective compound found in plants, animals and microorganisms. Accumulation of GB in higher plants is known to occur in response to various abiotic stresses, such as drought, high salinity and cold (Jagendorf and Takabe 2001, Rontein *et al*. 2002). Exogenous GB also enhances the tolerance of plants to various stresses (Ashraf and Foolad 2007, Chen and Murata 2008, Hoque *et al*. 2007, Mahouachi *et al*. 2012, Park *et al*. 2006). GB functions not only as an osmolyte but also as a molecular chaperon (Sakamoto and Murata 2002), and in plants it is synthesized from choline via betaine aldehyde (Sakamoto and Murata 2000). By manipulating this pathway, GB accumulation may occur in non-GB-accumulating plants. Interestingly, enhancement of this pathway using genetic engineering (GE) techniques has succeeded in conferring drought stress tolerance to a number of plant species (Cheng *et al.* 2013a). The betaine aldehyde dehydrogenase (BADH) is one of the GB bio-synthesis enzymes that catalyses betaine aldehyde in GB. A transgenic potato was generated with the *BADH* transgene from spinach driven by the stress-induced promoter *rd29A* from *Arabidopsis thaliana* (Zhang *et al.* 2011). BADH activity was abundant in all four transformants under stress. Using potted plants maintained in greenhouses, tolerance to drought and salinity stress was evaluated using growth performance and the electrical conductivity of leaves. The fresh weight of all transformants increased (17–29%) under stressful conditions compared to non-transformants (NT). Furthermore, there was a negative relationship ($r = -0.9495$) between BADH activity and the relative electrical conductivity in the leaves of the transformants (Zhang *et al*. 2011). This result demonstrates that BADH conferred drought and salinity stress tolerance to potato plants.

The choline oxidase (COD) does not exist in higher plants and it directly catalyses choline in GB. Transgenic potatoes were generated using the *codA* gene from *Arthrobacter globiformis* (rhizobacterium) driven by the oxidative stress-induced promoter *SWPA2* from sweet potato (Kim *et al*. 2003), with a transit peptide from the small subunit of Rubisco from tobacco (Ahmad *et al*. 2008). Two transformants were evaluated for the accumulation of GB and tolerance to stress (drought and salinity). GB accumulation observed in transformants was 3–5 times higher following methyl viologen (MV) treatment, and transformants showed better performance under drought and salinity stress compared to NTs. Potted plants in growth chambers were evaluated for drought tolerance by withholding water for 14 days. Transformants maintained higher water content and had over twice the biomass (dry weight) of NTs. In addition, the same transformants under in vitro conditions showed tolerance to MV based on ion leakage measurements (Ahmad *et al*. 2008). A further study has been published by the same group. Subsequently, they focused on the antioxidant system under drought conditions (Cheng *et al*. 2013a). Tolerance was evaluated using photosynthetic parameters, chlorophyll content and malonic dialdehyde (MDA) content. Potted plants in a growth chamber were subjected to 4 days drought (10% or 20% PEG) and 2 days rehydration. Transformants had more efficient photosynthetic systems, higher chlorophyll content and lower MDA content than NTs. Furthermore, the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), was observed to be higher in transformants than NTs. Transformants also showed better recovery from water deficiency than NTs. The studies detailed above indicate that accumulation of GB is a good strategy for conferring drought stress tolerance to potato.

Trehalose accumulation has been observed in bacteria, yeast and desiccation tolerant plants under stressful conditions (Iordachescu and Imai 2008); however, other plants are also known to accumulate small amounts of trehalose (Kondrak *et al*. 2011). Trehalose is also an osmolyte that has the ability to adjust osmosis and protect molecules (Jain and Roy 2009). That is synthesized from glucose-6-phosphate (G6P) and UDP-glucose via trehalose-6-phosphate (T6P), and trehalose and T6P are synthesized by T6P phosphatase (TPP) and T6P synthase (TPS), respectively. Accumulation of trehalose has been observed in a tobacco transformant containing a single yeast *TPS* (*TPS1*) gene (Holmstrom *et al*. 1996). Transgenic potato plants were generated containing the yeast *TPS1* gene driven by the stress-induced promoter *DS2* from potato (Stiller *et al*. 2008). Transgene expression was not induced by stress and expression levels were low in two independent lines. Although trehalose accumulation was not evaluated, water withholding was assessed in potted plants maintained in greenhouses. Both transformant lines showed higher relative water content and photosynthesis than NTs under drought conditions. Stomatal conductance was monitored in potted plants in the field under water withholding conditions and was observed to be lower in NTs than in transformants 3 days after drought treatment. At the beginning of the desiccation period, stomatal conductance was higher in NTs than in both transformant lines, possibly as a result of low stomatal density in transformants. Thus, trehalose accumulation was observed to confer drought tolerance to potato plants. However, transformants showed low stomatal density, which may cause low CO_2 fixation rates, leading to growth retardation under optimum conditions. An additional study was published using transcriptome analysis to investigate pleiotropy (Kondrak *et al*. 2011, 2012). A complex regulatory network, including transcriptional factors and other regulatory proteins, resulted in multiple phenotypic traits in transformants with the yeast *TPS1* gene.

Glucosylglycerol (GG) is a compatible solute typically found in moderate halotolerant cyanobacteria and some bacteria (Pocard *et al*. 1994, Reed and Stewart 1985, Roder *et al*. 2005). Of the terrestrial plant species, only *Myrothamnus flabellifolia*, one of the resurrection plants, is known to accumulate GG under drought conditions (Bianchi *et al*. 2006), and some members of Liliaceae constitutively synthesize GG (Kaneda *et al*. 1984). The GG-phosphate phosphatase/synthase (*ggpPS*) gene was transferred from *Azotobacter vinelandii* (rhizobacterium) to *Arabidopsis*. The transformant was observed to accumulate GG and exhibit stress tolerance (Klähn *et al*. 2009). To confer drought and salinity tolerance to potato, potato plants were generated expressing the *ggpPS* gene under control of the constitutively expressed cauliflower mosaic virus *35S* promoter (*35S*) or the *rd29A* promoter (Sievers *et al*. 2013). Although both transformants accumulated GG in leaves, only the *rd29A* transformants accumulated GG in tubers. In greenhouse experiments, a number of lines from both transformants exhibited improved shoot growth compared to NTs under drought and salinity conditions.

Drought tolerance has also been conferred to potato using other approaches. To understand the synthetic pathway of fructans, transformants with *sucrose: sucrose 1-fructosyltransferase* (*1-SST*) and *fructan: fructan 1-fructosyltransferase* (*1-FFT*) genes from the globe artichoke were generated and were able to synthesize the high molecular weight carbohydrate inulin (Hellwege *et al*. 2000). Inulin has been defined as a type of polysaccharide consisting of sucrose with β(2-1)-linked fructose (fructan) chains. It has been suggested that fructans contributes to the osmotic stress tolerance of organisms (Hendry 1993, Park *et al*. 1999), possibly via its stabilising effects on membranes and proteins (Fricke and Pahlich 1990, Vereyken *et al*.

2003). It has been suggested that inulin also plays a role in conferring drought tolerance (Hendry 1993, Pilon-Smits *et al*. 1995). Accumulation of proline was observed to be suppressed in *1-SST/1-FFT* transformants under water deficit conditions; however, the turgor potential in leaves was reduced (Knipp and Honermeier 2006). Fructan is thought to contribute to osmotic control in potato transformants. As reported in the other study, repression of formate dehydrogenase (FDA) enhances accumulation of proline under drought stress (Ambard-Bretteville *et al*. 2003). This transformant had reduced levels of the FDA protein and accumulated formate in leaves and tubers, and under drought stress, the transformant accumulated proline more rapidly than NTs. However, there was no description of drought tolerance in this study.

The YUCCA family is known to contribute to auxin bio-synthesis in plants. A rice mutant, *CONSTITUTIVELY WILTED1*, was deficient in the YUCCA homologue (Woo *et al*. 2007) and the activation tag line of *YUCCA7* in *Arabidopsis* demonstrated drought tolerance (Lee *et al*. 2011). Drought tolerance by over-expression of *AtYUC6* in potato was evaluated in 4-month-old potted plants in greenhouses by monitoring recovery after withholding water for 18 days (Kim *et al*. 2013). Although NTs wilted, transformants survived and exhibited higher water content in leaves and lower reactive oxygen species (ROS) content with increased expression of *CuZnSOD* compared to NTs. Based on these characteristics, this transformant may also be tolerant of other abiotic stresses.

The studies discussed above demonstrate that drought tolerance is not easily controlled and various factors are intricately involved. It has been reported that biotic stress related proteins are also associated with abiotic stresses, such as osmotic and salinity stress. Pathogenesis-related proteins (PR proteins) are induced by pathogen infection or wounding, while PR-10 proteins are also induced by abiotic stresses, such as drought, salinity or oxidative stress (Liu and Ekramoddoullah 2006). Based on the results of proteomic analyses, the PR-10a protein in potato was induced by osmotic or salinity stress, and over-expressing callus of this gene indicated osmotic and salinity tolerance in suspension culture (El-Banna *et al*. 2010). This transformed callus showed low oxidised glutathione content and high proline accumulation under 0.5 M sorbitol or 0.32 M NaCl containing medium compared to NT callus. From these studies, it is evident that various genes contribute to confer drought tolerance to potato.

Salinity

Potato is a moderately salt-sensitive crop and is able to withstand soil salinity up to approximately 2.0 dS/m (Mass and Hoffman 1977). Dependent on the soil type, EC values reached 5.8 dS/m following 100 mM NaCl irrigation in our experiment. Salt affected soil has been shown to have a negative effect on potato crops: salinity at 2.0 g/L delays seed germination, retards growth and results in reduced tuber yield with low quality (Katerji *et al*. 2003), and salinity of 5.9 dS/m decreases potato production by half (Kotuby-amacher *et al*. 2000). Irrigated land is gradually is suffering from salinity damage and combating salinity stress is a pressing issue for world agriculture, including potato production.

Salinity damage results in two different adverse effects on plant cells, ionic stress and osmotic stress. Osmotic regulation confers salinity tolerance to plants: a high solute concentration (molar concentration) causes water to remain in the cytoplasm and osmoprotectants contribute to maintaining the function of proteins. Mannitol is an osmoregulatory substance typically used in experiments; it is found in many plant species and is known as the most abundant sugar alcohol in nature (Patonnier *et al*. 1999, Prabhavathi and Rajam 2007, Shen *et al*. 1997). Potato plants do not naturally accumulate mannitol, so transformants with the *mannitol-1-phosphate dehydrogenase* (*mtl*D) gene from *E. coli* were generated to confer salinity tolerance to potato (Rahnama *et al*. 2011). Accumulation of mannitol in these transformant lines was abundant under 50 mM NaCl salinity conditions in vitro and in hydroponic culture; however, it was decreased dependent on salinity strength in high NaCl concentrations. Growth parameters were assessed in a hydroponic culture system to evaluate tolerance. In 100 mM NaCl conditions, the dry weight of NT shoots was reduced by 76.5%; however, in the elite transformant line the dry weight of shoots was only reduced by 17.3%. Thus, these results suggest that the bacterial *mt1*D gene may confer the salinity tolerance to potato. Accumulation levels of mannitol were not enough to osmotic adjustment in these transformants, mannitol may function as an osmoprotectant in this case.

Proline is known to be an important stress responsive substance and is also thought to be an osmoprotectant. The *pyrroline-5-carboxylate synthetase* (*P5CS*) gene from *Arabidopsis* was introduced into potato driven by the *35S* promoter (Hmida-Sayaria *et al*. 2005). Under 180 mM NaCl conditions in vitro, proline accumulation in leaves was over five times higher in the elite transformant line than in NTs. The elite transformant line also showed higher tuber production than NTs under 100 mM NaCl conditions using potted plants in a greenhouse. Improvements in proline bio-synthesis resulted in enhanced salinity tolerance in potato. In addition to the above studies, transfer of the *glyceraldehyde-3-phosphate dehydrogenase* (*GPD*) gene from oyster mushrooms into yeast resulted in the transfer of salinity tolerance (Jeong *et al*. 2000). This group also tried to introduce this gene, driven by the *35S* promoter, into potato (Jeong *et al*. 2001). They evaluated salinity tolerance of potato in a growth chamber by cultivating plants in fresh water for 10 days following 2 M NaCl treatment for 12 h. Although NTs were wilted, six independent transformant lines survived. The mechanism of tolerance was not mentioned; however, transfer of the *GPD* gene may confer salinity tolerance to potato.

Although one strategy for adapting to salinity is similar to that used for adapting to drought, another strategy is to remove Na^+ from the cytoplasm. Extrusion of Na^+ from cells is carried out by Na^+/H^+ antiporters in the plasma membrane and compartmentation into vacuoles is undertaken by Na^+/H^+ antiporters in the vacuolar membrane. However, exclusion of ions from cells is not sufficient to protect plants against high salinity because the excluded ions remain in the apoplast resulting in osmotic stress component (Blumwald *et al*. 2000). A transgenic potato was generated with a *vacuolar* Na^+/H^+ *antiporter* (*HvNHX2*) gene from barley driven by the *35S* promoter (Bayat *et al*. 2010). Although NTs were unable to survive 200 mM NaCl in vitro, five of the six transformant lines survived. Three elite transformant lines showed higher K^+ content compared to NTs under 100 and 150 mM NaCl conditions. On the other hand, another group reported about transformants with the *AtNHX1* gene from *Arabidopsis*, and evaluated the performance of eight lines using potted plants in a greenhouse (Wang *et al*. 2010). Salt concentrations that resulted in a 50% reduction in tuber yield (YR_{50}) and 50% plant survival (LD_{50}) were employed as tolerance indicators in this work. The elite transformant lines indicateed over twice higher in both indicators compared to NTs. Furthermore, tolerant lines maintained higher chlorophyll content and had lower proline accumulation than NTs. These results indicate that tolerant lines may lessen the salinity stress by confining Na^+ to vacuole wells. However, tolerant lines did not show a remarkable increase in Na^+ content in leaves under high salt concentrations. These studies demonstrate that both strategies work to confer salinity tolerance in potato. These above studies demonstrate that both osmotic regulation and removal of Na^+ work to confer salinity tolerance in potato.

Low temperature

Potato plants like cool climates, thus, the commercial cultivation of potato is suitable for high altitude or highland regions. As a consequence, potato production often suffers from low temperature stress, especially just after planting or just before harvesting. The display of a low temperature tolerant phenotype is important, and introgression of this phenotype has been an urgent requirement in potato production. Low temperature causes reduction of various enzymatic activities and of floridity of bio-membrane. Under freezing temperature, ice formation occurs in intercellular spaces. This leads to dehydration in cytoplasm (Sinha 2004).

One strategy for adapting to low temperatures is the desaturation of fatty acids in the bio-membrane. Unsaturated fatty acids are known to increase in the bio-membrane of various plants species exposed to low temperatures (Clarkson *et al*. 1980, Horvath *et al*. 1981, Kodama *et al*. 1995, Smolenska and Kuiper 1997) and they are also associated with chilling or freezing tolerance (Miquel and Browse 1992, Miquel *et al*. 1993, Quinn *et al*. 1989). Desaturation of fatty acids in constitutive lipids increases the fluidity of the membrane by depressing the solidification point and maintains the function of the bio-membrane, even at low temperatures. Desaturases involved in the desaturation of fatty acids can be classified into two types; delta (Δ) and omega (ω). Δ and ω notations indicate the position of the carbon bonds from the carbonyl carbon and the methyl end, respectively (Napier *et al*. 1999). There are many reports that transformation of desaturases confers low temperature tolerance in various plant species, such as tobacco (Kodama *et al*. 1994) and tomato (Yu *et al*. 2009). In potato, the *Δ12-desaturase* gene from cyanobacteria was introduced to plants and its ability to improve low temperature sensitivity was evaluated (Amiri *et al*. 2007). From the analysis of in vitro plants, all four lines had reduced MDA content under –7°C conditions, and three of the four lines showed increased unsaturated fatty acid content. They concluded that introduction of the *Δ12-desaturase* gene from cyanobacteria conferred low temperature tolerance to potato. Subsequently, they focused on only three lines that increased unsaturated fatty acid content. From the evaluation of a four-month-old in vitro cultivation at 8°C, all three transformants showed cold stress tolerance traits, such as viability, less visible damage and high microtuber production (Amiri *et al*. 2010). Using a part of same transformant lines (probably), generation of oxidative molecules under low temperature was evaluated in in vitro (Demin *et al*. 2008). Following exposure to low temperature, the presence of oxidative molecules, including superoxide anion and hydrogen peroxide, and subsequently a decrease in MDA content, were observed in transformant lines compared to NTs. However, activity of SOD was lower in transformant lines than that in NTs, suggesting that the generation of oxidative molecules was suppressed in the *Δ12-desaturase* transformants. On the other hand, ω9-desaturase from *Solanum commersonii* was transformed to potato (De Palma *et al*. 2008). *S. commersonii* is a wild relative of *S. tuberosum* and has high abiotic stress tolerance, including cold, drought and salinity tolerance. The transformant lines had a C18:1 Δ11 fatty acid that was not found in the vector control (VC) transformants. The freezing tolerance of the potted plants was evaluated in a growth chamber by ion leakage. The LT_{50} of transformants decreased by approximately 2°C after cold acclimation (De Palma *et al*. 2008). Although these results are not from natural conditions, they imply that desaturation of fatty acids in membranes may confer a certain level of cold tolerance to plants.

Another strategy used by plants against low temperature stress is osmotic regulation. A high solute concentration (molar concentration) can enable plant cells to retain water or prevent ice formation. Generally, ice formation begins in the apoplastic area where solute concentration is the lowest. As ice formation expands in the apoplast, water is gradually withdrawn from the cytosol and cell desiccation progresses (Steponkus and Webb 1992). To prevent ice formation, yeast invertase was introduced to potato. Since over-expression in leaf or cytosolic localization of this gene (product) caused low productivity of tuber (Heineke *et al.* 1992, Sonnewald *et al.* 1997), the transgene was driven by the tuber specific promoter patatin *B33* and fused with the protease inhibitor II leader peptide for apoplastic enzyme localisation (Deryabin *et al*. 2003). Using in vitro plants, invertase activity, sugar content and low temperature tolerance (measured by MDA content) were investigated. Invertase activity doubled, especially that of the acidic type, and sugar content, such as sucrose, also increased in the apoplastic and the cytosolic fractions under control condition. After exposing plants to –9°C for 20 min, MDA content was 1.5 times higher than that under non-stress condition in VC plants; however, the level of MDA was stable in transformants. An extended low temperature treatment at 5°C for 5 days also revealed that MDA content was lower in transformants than that in VC plants. This work used the *B33* promoter driven transformant whose transgene is thought to be abundant in tubers. Readers may also be interested in whether the transgene contributes to the enhancement of low temperature tolerance in leaves. The author also evaluated *B33* promoter activity in leaves, stems and roots using the *B33* promoter *GUS* transformant of potato. Of the three tissues evaluated, GUS activity was highest in leaves, and was approximately 10% of the activity observed for the *35S* promoter GUS in leaves. Based on this result, some phenotypes, including bio-chemical properties, are thought to have been caused by weak expression of the transgene in potato leaves. Under natural conditions, low temperature tolerance may be more essential in aerial sections of the plant rather than the tubers. It is expected that high expression of this invertase in aerial sections of the potato plant may confer increased tolerance of low temperatures. Based on the results of the above studies, it is evident that various genes may enhance low temperature tolerance in potato. However, there are no reports conferring cold acclimation to potato using GE techniques as detailed mechanisms and the genes responsible remain to be elucidated.

In addition to the above examples, I would like to highlight a previous study that transferred the synthetic antifreezing protein (AFP) to potato (Wallis *et al*. 1997). AFP inhibits ice growth and recrystallization, and is effective in protecting against frost damage at concentrations several orders of magnitude lower than freezing point depression by mol concentration (Carpenter and Hansen 1992, De Vries 1986). Performance of an AFP transformant was evaluated by measuring electrolyte release under freezing temperatures. The decrease in electrolyte release was observed to be dependent on the accumulation of AFP at –2.5 and –3.0°C. Synthetic AFP was regarded as conferring low temperature tolerance to potato.

Oxidative Scavengers

Environmental stresses such as drought, salinity and cold are known to enhance the accumulation of ROS in plant cells. Various types of ROS result in the oxidation of many cellar components and lead to oxidative destruction of the cell (Asada and Takahashi 1987, Mittler 2002). Enhancement of the ROS scavenging system is expected to confer tolerance to various types of stresses in plants. Scavenging of ROS in plant cells is carried out via enzymatic and non-enzymatic mechanisms. A network of low molecular weight antioxidants, such as ascorbate (AsA) and glutathione (GSH), and antioxidative enzymes, such as SOD and CAT, are important for preventing and controlling the dangerous effects of ROS (Noctor and Foyer 1998).

Improving the stress tolerance of plants using low molecular weight antioxidants could be performed by increasing the production rate of reduced forms of GSH and AsA, and the enlargement of either pool. AsA is a crucial antioxidant molecule that scavenges free radicals and protects from ROS generated by adverse environmental conditions. AsA can directly scavenge free radicals (Halliwell and Gutterridge 2000) and is an important electron donor for H_2O_2 via AsA peroxidase (APX) (Noctor and Foyer 1998). On the other hand, GSH is involved in detoxification of xenobiotic compounds (Noctor and Foyer 1998, Potters *et al*. 2002) and redox regulation in the cell (Noctor *et al*. 1998). GSH also reduces oxidised AsA (dehydroascorbate: DHA) to AsA. GSH is converted to glutathione disulfide (GSSG) by scavenging oxidative molecules. GSSG is catalysed to GSH by GSH reductase (GR) in the NADPH-dependent manner. DHA is reduced to AsA by dehydroascorbate reductase (DHAR). To enhance the ratio of AsA, the *DHAR1* gene from *Arabidopsis* driven by the *35S* promoter was introduced into potato (Eltayeb *et al*. 2011). The transformants showed higher MV tolerance, evaluated by ion leakage of leaf discs, and higher chlorophyll content compared to NTs. Furthermore, these transformants grew better under salinity and drought conditions compared to NTs. In addition to this work, same group also tried to enhance the ability of GSSH reduction by introducing the *GR1* gene from *Arabidopsis*, which is a cytoplasmic isoform, driven by the *35S* promoter (Eltayeb *et al*. 2010). The transformants with the *GR1* gene had 6.5 times higher GSH content, 5.8 times higher GR activity, and showed higher relative AsA content compared to NTs. Improved tolerance was observed as increased root growth in a heavy metal ion and low ion leakage from leaf discs assessed by MV treatment. Based on these results, high levels of the reduced forms of GSH and AsA may confer various abiotic stress tolerances to potato.

Furthermore, there are five recent reports from a Korean group demonstrating enhancement of the bio-synthesis of AsA. The transformant was generated with the *D-galacturonic acid reductase* (*GalUR*) gene from strawberry driven by the *35S* promoter (Hemavathi *et al*. 2009). Three independent transformant lines showed high AsA accumulation with high GalUR activity compared to NTs. Two elite lines indicated a reduction in leaf disc breaching following MV treatment (~10 mM), NaCl (~600 mM) and mannitol (~300 mM). Furthermore, the in vitro growth performance of these two lines was better than that of NTs in NaCl (~200 mM) containing media. Similar results were observed in over-expression of the *L-gulono-γ-lactone oxidase* gene in potato (Hemavathi *et al*. 2010). Biochemical properties, such as ROS scavenging enzymatic activity and proline or MDA content, of one line of the *GalUR* transformants were also further analysed during MV, NaCl or ZnCl treatment (Hemavathi *et al*. 2011). SOD, CAT, APX, DHAR and GR activity was higher in transformants than in NTs under stressful conditions. Levels of reduced forms of AsA and GSH were higher in transformants than in NTs under three different stressful conditions and control conditions. Furthermore, under stressful conditions, microtuber production was over twice as high, with associated increased proline content and reduced MDA content, in transformants than in NTs. It is thought that enhanced accumulation of AsA causes upregulation of the antioxidant system, improving tolerance to various abiotic stresses. Single gene over-expression of *GalUR* resulted in alteration of various antioxidant systems. The *GalUR* transformant maintained photosynthetic performance and showed improved productivity under salinity conditions (Venkatesh *et al*. 2012). The same group also focused on metheylglyoxal (MG) accumulation (another cytotoxic compound) under salinity conditions (Upadhyaya *et al*. 2011). MG concentration is regulated by the glyoxalase system, which is dependent on GSH concentration (Yadav *et al.* 2005a, 2005b). In contrast to NTs, transformants maintained MG levels under salinity conditions by increasing the activity of two glyoxalases.

Same Korean group tried to suppress evolving oxidative molecules from photosystem II. They focused on the manganese-stabilising protein (MSP), which is a key component of the oxygen-evolving complex (Williamson *et al*. 2011), and generated both sense and antisense transformants with the *MSP* gene from *S. tuberosum* driven by the *35S* promoter (Gururani *et al*. 2012). MSP sense plants had almost same the properties (morphology, tuber yield, photosynthetic performance) as NTs. However, higher tuberisation frequency and higher tuber yield, with increased total soluble sugars, was observed in antisense plants compared to NTs and sense plants. Furthermore, antisense plants had greater photosynthetic activity and lower oxygen evolution compared to NTs and sense plants. Proline and AsA content was also higher in antisense plants than in NTs and sense plants. Consecutively using these antisense plants, they evaluated abiotic stress tolerance, including as salinity (200 mM NaCl), heavy metal ions (20 mM $ZnCl_2$) and drought (200 mM mannitol) (Gururani *et al*. 2013). In addition to proline and AsA content, α-tocopherol and ABA levels also increased in antisense plants compared to NTs. Levels of oxidative scavenging enzymes, such as APX, CAT, SOD and DHAR, were also higher in antisense plants than in NTs under stressful conditions. Using potted plants in greenhouses, NTs showed more severe damage under the above stressful conditions compared to antisense plants. Suppression of MSP appears to confer higher performance to potato plants under stressful conditions and situations with high scavenging ability.

Antioxidative enzymes, such as SOD and CAT, are also important for direct scavenging of ROS. SOD catalyses dismutation of the superoxide radical (O_2^{*-}) into H_2O_2 and O_2 (Asada 1999), and SOD contributes to an organism's primary defence against oxidative molecules. Cytosolic copper-zinc (Cu/Zn) SOD from *Potentilla atrosanguinea* (Himalayan cinquefoil) was transformed to potato driven by the *35S* promoter (Pal *et al*. 2013). Potted plants evaluation revealed that the highest SOD activity line of these transformants indicated higher net photosynthetic rate and stomatal conductance under control and salinity conditions compared to NTs. Drought conditions reduced water potential, photosynthetic activity and chlorophyll content, but increased proline and O_2^{*-} content in NTs. However, these changes were diminished in transformants. Another Korean group generated potato transformants (SSA) with the *Cu/ZnSOD* gene from *Manihot esculenta* (Cassava) and the *APX* gene from *Pisum sativum* driven by the oxidative stress inducible *SWPA2* promoter (Tang *et al*. 2004) with a transit peptide for chloroplast (Tang *et al*. 2006). APX reduces H_2O_2 to H_2O utilising ascorbate as an electron donor. These SSA lines indicated MV tolerance by bleaching of leaf discs. The MV and high temperature tolerances of two elite lines from SSA transformants were subsequently evaluated using potted plants in a growth chamber. Both lines indicated diminishing MV damage, including visible features and a reduction in dry weight, and a reduction in chlorophyll content. These two transformant lines also showed better growth and maintained photosynthetic activity under high temperature conditions. However, it is difficult to discuss simultaneous effects of SOD and APX in the SSA line as the work on a single transformation of SOD was done by another group using different cultivars and different evaluation methods. Furthermore, they introduced the *codA* gene, with a transit peptide for chloroplast, from rhizobacterium driven by the *SWPA2* promoter to the SSA transformant (Ahmad *et al*. 2010). These transformants (SSACs) had three transgene products (SOD, APX and GB) induced by oxidative stress in chloroplasts. Two lines of the SSAC transformants indicated better growth performance than NTs and SSA transformants under saline (200 mM NaCl) and drought (withholding water for 14 days) stress conditions.

The same group also tried to confer abiotic stress tolerance to potato by transferring the *nucleoside diphosphate kinase 2* (*NDPK2*) gene from *Arabidopsis* (Tang *et al*. 2008). NDPK is a housekeeping enzyme that maintains the levels of (d)NTPs. Recently, it was suggested that this enzyme is also associated with signal transduction in oxidative stress (Otero 2000). Transformants generated with the *AtNDPK2* gene, driven by the *SWPA2* or the *35S* promoter, were evaluated for their tolerance to various stressful conditions using potted plants. Following treatment with 250 mM MV in a greenhouse, transformants showed 30–50% less visual damage than NTs. Furthermore, transformants exhibited reduced damage under high temperature (42°C) and salinity (80 mM NaCl) conditions compared to NTs. It is thought that the expression of *NDPK2* caused upregulation of *APX* and consequently enhanced various abiotic stress tolerances. Subsequently, this group transformed the *SWPA2:AtNDKP2* construct into an SSA transformant (Tang *et al*. 2006) and generated new transformants that possessed three transgenes (SSAN) to evaluate the contribution of multiple genes (Kim *et al*. 2010). Although the activity of products from transgenes was increased in each transformant under oxidative stress conditions, APX may be upregulated in transformants with the *NDKP2* transgene. Furthermore, CAT activation was clearly observed in the transformant with *NDKP2.* The SSAN transformants showed high activity of three major oxidative scavenging enzymes (SOD, APX and CAT). Based on an ion leakage evaluation treatment using 3 mM MV, SSAN transformants showed significantly higher tolerance than other transformants and NTs. Other parameters also indicated high tolerance in SSAN transformants under oxidative and high temperature stress conditions. Although simultaneous effects were mentioned in this work, there were no statistically significant differences between SSA and SSAN, except for ion leakage evaluation under oxidative stress.

The studies discussed provide knowledge on oxidative stress tolerance and future breeding for abiotic stress tolerance in, not only potato, but also higher plant species. It is expected that simultaneous effects of transgenes will be evaluated in field trials. In addition to the various studies discussed above, the same group generated transformants with *2-cystein peroxiredoxin* (*2-Cys Prx*) genes from *Arabidopsis* driven by the *SWPA2* or the *35S* promoter, and evaluated their tolerance under oxidative and high temperature stress conditions using potted plants (Kim *et al*. 2011). The 2-Cys Prx is an antioxidative enzyme that removes peroxides and protects the photosynthetic membrane from oxidative damage (Dietz 2003). Under oxidative stress treatment using 300 mM MV, photochemical yield and chlorophyll content were reduced by 60% in NTs but by only 20–35% in transformants compared to control conditions. Moreover, photochemical yield after treatment at 42°C for 10 h was reduced by 31% in NTs, but by only 7% in an elite transformant line compared to before treatment. These transformants had reduced damage to photosynthetic mechanisms under stressful conditions and showed high oxidative and high temperature stress tolerance.

Another group attempted to generate six types of transformants using four types of transgenes (Waterer *et al*. 2010) driven by the stress inducible *COR78* promoter from *Arabidopsis* (Thomashow 1999) or the *35S* promoter. The four transgenes were mitochondrial *MnSOD* (*SOD3:1*) from wheat, *dehydrin 4* (*DHN 4*) from barley, a cold inducible transcriptional factor *DREB/CBF* from canola and the stress associated gene *ROB5* belonging to the LEA group 3 protein from bramegrass. The *COR78* promoter was used for all four genes, and the *35S* promoter was used for the *SOD* and *ROB5* genes (Waterer *et al*. 2010). Based on results using potted plants in a growth chamber, all transgenes were found to enhance high temperature (44°C) tolerance, especially the *COR78:DHN4* and *COR78:ROB5* transformants. Under low temperature conditions (10°C), the *35S:SOD3.1* transformant grew better than the NTs. The freezing temperatures, measured using ion leakage, were also reduced in *COR78:SOD3.1*, *35S:SOD3.1* and *35S:ROB5* transformants. These experiments were also carried out four times in the field (2001, 2003, 2005 and 2006). Evaluation in field conditions is quite different from that under artificial conditions as the weather is unstable and uncontrollable. In 2005, the weather differed from the other years, and was relatively cooler and wetter. The tuber yield of almost all the transformants indicated a reverse tendency in 2005 compared to the other years. The overall average of tuber yield taken across the four years is apt to extinguish the stress tolerance trait of transformant due to differences in weather tendency conditions (e.g. warm or cool, dry or rainy). However, *COR78* driven transformants, except DREB/CBF, indicated improved productivity compared to NTs. This work indicates that GE techniques may contribute to breeding programs using abiotic stress tolerance for practical purposes.

Transcriptional factors

All the examples discussed above were aimed at altering single or multiple genes. In contrast, there are some studies that use transcriptional factors (TFs), which influence the expression of various downstream genes. TFs regulate the expression of downstream genes by binding to specific elements of target genes (Lee *et al*. 1999, Ramanjulu and Bartels 2002). When plants detect stress in the environment, the responsible TF is induced, which controls the necessary gene set for the corresponding physiological responses. Since this mechanism is a native physiological response in plants, it has been suggested that transformation of TFs can mimic natural situations in which plants are exposed to a stress (Pino *et al*. 2007).

In *Arabidopsis*, identification and characterisation of abiotic stress related TFs was carried out, and of these, drought-responsive element binding factors (DREB) and C-repeat binding factors (CBF) have been well studied (DREB1A-B-C corresponds to CBF3-1-2, respectively; Gilmour *et al*. 1998, Liu *et al*. 1998, Stockinger *et al*. 1997). These TFs have the APETALA2 (AP2)/ethylene response factor (ERF) domain composed of approximately 60 amino acids (Sakuma *et al*. 2002). To confer abiotic stress tolerance to potato, the *AtDREB/CBF* gene was transferred to potato. There are several studies of *AtDREB/CBF* transformants mainly conducted by two different groups. One group employed the *rd29A* promoter (Behnam *et al*. 2006, Celebi-Toprak *et al*. 2005) as it had already been revealed that over-expression of *AtDREB1A* by the *35S* promoter resulted in growth retardation in *Arabidopsis* plants under control conditions (Kasuga *et al.* 1999). Using 4–6 week-old cuttings propagated potato on soil, transformants were able to survive 2 M NaCl (Behnam *et al*. 2006). A positive relationship was observed between tolerance levels and integrated copy numbers or expression levels of the transgenes. However, transformant lines with high integrated copy numbers did not follow this relationship. Furthermore, this group also evaluated freezing tolerance and compared this to tolerance of salinity conditions (Behnam *et al*. 2007). Evaluation of freezing tolerance was carried out at –20°C for 1 h in vitro after acclimation at 4°C. Expression levels of transgenes were positively correlated with survival rate after freezing, except one transformant line with high transgene expression (D138). Salinity and freezing tolerances were expected to show the same tendency in all transformant lines; however, some lines exhibited only salinity or freezing tolerance. D138 is a typical example and this line was categorized as a salinity tolerant line with high expression of transgene. Differences in tolerance may be due to positional effects, such as insertion mutation or alteration of epigenetic regulation by the transgene. These various lines may help to clarify downstream regulation or help identify responsible genes using microarray analyses. In addition to salinity and freezing evaluations, these transformants were also evaluated for drought tolerance in vitro (Huynh *et al*. 2014). Evaluation of drought tolerance for intact plants was carried out in liquid culture at –1.8 MPa PEG8000 for 9 days and consecutively without PEG for 3 days. The expression levels of transgenes were also positive correlated to drought tolerance levels. From these studies, AtDREB1A was observed to enhance not only freezing, but also drought and salinity tolerance in potato.

Another group has also generated transformants with all three *DREB/CBF* genes driven by the *35S* or the *rd29A* promoter independently (Pino *et al*. 2007). Among these six transformants, *AtCBF2* driven by both promoters did not show freezing tolerance. However, *AtCBF1* and *AtCBF3* driven by both promoters improved their LT_{50} values by approximately 2°C, evaluated using ion leakage analysis in potted plants in a growth chamber. While, these *35S* type transformants showed some negative phenotypes, such as plant stunting, delayed flowering and reduction of tuber production, the *rd29A* type transformants displayed improvements of these negative phenotypes. This work elucidated that *AtCBFs,* except *AtCBF2,* enhance freezing tolerance in potato, and directly indicated that in potato the *rd29A* promoter may improve defects caused by constitutive expression of the *AtCBF1* and *AtCBF3* genes. Furthermore, the *35S:AtCBF1* gene also enhanced freezing tolerance by approximately 2°C in *S. commersonii*, which is a cold tolerant wild relative of *S. tuberosum* (Pino *et al*. 2008). However, only the freezing tolerance of the transformants was evaluated. Transformant lines with the *AtDREB1B* gene driven by the *35S* promoter were evaluated by a different group from the two mentioned above (Movahedi *et al*. 2012). Using potted plants, in addition to freezing stress tolerance, transformant lines showed drought tolerance. Based on these results, it is expected that *AtDREB1B* will also enhance freezing, drought and salinity tolerance in potato, similar to *AtDREB1A*.

Other transcriptional factors have also been transformed into potato. One study has evaluated transformants with the *pathogen and freezing tolerance-related protein 1* (*CaPF1*) gene from *Capsicum annuum* (pepper) driven by the *35S* promoter (Youm *et al*. 2008). This gene encodes the AP2/ERF-type TF that was isolated by inoculation with a bacterial pathogen (*Xanthomonas axonopodis*) and was found to affect the expression of genes that contain either a GCC to a CRT/DRE box in their promoter regions in over-expression of *Arabidopsis* (Yi *et al*. 2004). Transformant lines in potato indicated enhanced tolerance to freezing, heat, drought, oxidative stress and heavy metal ions. Furthermore, tuber formation in vitro was retarded in transformant lines compared to NTs. Another study evaluated transformants with the *IbMyb1* gene from sweet potato driven by the *SWPA2* promoter, which encodes the R2R3-type Myb TF (Cheng *et al*. 2013b). The R2R3-type Myb TF is known to be involved in secondary metabolism and responds to biotic and abiotic stresses (Dubos *et al*. 2010, Stracke *et al*. 2007). The IbMyb1 regulates anthocyanin bio-synthesis in tuberous roots (Mano *et al*. 2007). Using potted plants under 400 mM NaCl conditions, two independent transformant lines exhibited better root growth than NTs and contained higher levels of secondary metabolites, such as total phenols, total flavonoids and anthocyanin. Salinity, UV-B and drought stress induced the expression of flavonol and anthocyanidin biosynthesis genes, which cooperated with *IbMyb1* expression. Transformants also showed higher radical scavenging activity than NTs. Over-expression of *IbMyb1* was observed to affect secondary metabolism, and consequently may improve abiotic stress tolerance in potato.

The examples discussed above indicate that certain heterogenic TFs could enhance abiotic stress tolerance in potato. Recent studies have attempted to isolate orthologue genes for the TFs discussed above in potato, and to confirm their functions in potato using GE techniques. Almost all of these studies confirmed abiotic stress tolerance in the transformants and described properties of the transgene products as the TF, such as nuclear localisation, binding ability to specific DNA sequences or regulation of downstream genes. Here, the discussion focuses on enhancement of abiotic stress tolerance. The *Ethylene responsive element binding protein 1* (*StEFEBP1*) gene from *S. tuberosum* was isolated using a cold induced cDNA pool, and then its TF was characterized by constitutive over-expression via the *35S* promoter in potato (Lee *et al*. 2007). Under cold conditions, yield increased by approximately 50% in the elite transformant line compared to NTs. Growth performance was also better in all transformant lines than in NTs under salinity conditions (75 mM NaCl) in vitro. The same group generated transformants with the *StMyb1R-1* gene, which encodes the R-1 type MYB-like TF, previously isolated as a stress inducible gene (Lee *et al*. 2007). The performance of transformants was evaluated under drought conditions, using potted plants in a greenhouse, by withholding water for 15 days and re-watering for 7 days (Shin *et al*. 2011). During the stress treatment, transformants displayed less visual damage with reduced water loss and higher survival rate than NTs. Sensitivity for ABA also increased in transformants. Under control conditions, obvious negative traits did not find from the picture in this report (Shin *et al*. 2011). Another group attempted to isolate the *DREB* orthologue from *S. tuberosum* based on information from a database, and generated transformants to characterize the isolated genes. They generated transformant lines with the *StDREB2* gene driven by the *35S* promoter, and evaluated their salinity tolerance (Bouaziz *et al*. 2012). Transformant lines showed better growth in vitro under saline conditions (200 mM NaCl for 40 days), and had less water loss and higher proline accumulation than NTs (100 mM NaCl for 20 days). Furthermore, transformants were also generated with the *StDREB1A* gene driven by the *35S* promoter, and their tolerance of salinity and drought was evaluated (Bouaziz *et al*. 2013). Transformant lines had less water loss and higher proline accumulation than NTs under saline conditions (100 mM NaCl for 20 days). Using potted plants in a greenhouse, elite lines exhibited better growth of their aboveground portions under saline (100 mM NaCl for 20 days) and drought conditions (withholding water for 25 days). As in the examples above, identified TFs have been shown to enhance abiotic stress tolerance in potato. It is expected that these efforts will contribute to our understanding of the tolerant mechanisms used in potato against abiotic stress.

The *35S:AtCBF1* construct has also been transformed into *S. tuberosum* and *S. commersonii*, and the two transformant lines were compared to each other (Pino *et al*. 2008). While this study was partially introduced in the previous section, there were a few differences between the two species. *S. commersonii* is known to exhibit cold acclimation (Chen and Li 1980, Costa and Li 1993) and thickening of the leaves following exposure to low temperatures (Chen *et al*. 1977, Estrada 1982, Palta and Li 1979). Cold acclimation was not conferred to *S. tuberosum* by over-expression of the *AtCBF1* gene, while increased leaf thickness was observed in both species. The cold tolerant property of *S. commersonii* is thought to consist of a complex of traits that are not controlled by a single TF. Accumulation of similar studies will advance our understanding of cold tolerance and cold acclimation mechanisms in *S. commersonii*.

Study of stress tolerance in *S. commersonii*

S. tuberosum can endure temperatures of –3°C and does not have the ability to acclimate to cold conditions. Conversely, *S. commersonii* can endure temperatures of –5°C before acclimation and can survive at –11°C after acclimation (Chen and Li 1980, Costa and Li 1993). Molecular analyses of *CBF* in *S. commersonii* were performed. First, transcriptome analyses using *S. tuberosum* and *S. commersonii* were conducted under low temperature conditions (2°C) or by transformants with *35S:AtCBF3* (Carvallo *et al*. 2011). This work revealed that a cold regulated gene induced at a similar degree and with similar kinetics exists in both *S. tuberosum* and *S. commersonii*. However, considerable differences were observed in the gene sets composed of the cold transcriptome and CBF regulons of these two species. It was suggested that these differences contribute to the differences in freezing tolerance of the two species, including cold acclimation. Additionally, the genetic structure of *CBFs* have been investigated and compared among *Solanum* species and *Arabidopsis* (Pennycooke *et al*. 2008). Gene clusters of CBF (*CBF3-CBF1-CBF2* cluster) were maintained among investigated *Solanum* species and *Arabidopsis*, with some deletion or duplication. In particular, *S. tuberosum* and *S. commersonii* had one more *CFB2* (remnant *CBF2* in *S. commersonii*) in adjacent in *CBF2*. Although all *CBFs* in *Arabidopsis* were induced by cold, only *CBF1* showed cold inducible properties in the *Solanum* species. Furthermore, cold inducible *CBF4* existed on another locus of *S. tuberosum* and *S. commersonii*. *S. tuberosum* had *CBF5* closely linked to *CBF4*, and it was suggested that the *CBF5-CBF4* cluster resulted from duplication of the *CBF3-CBF1-CBF2* cluster. DNA motifs in up-stream regions of *CBF1* were found in *CBF4* in *Solanum* species, which were conserved in all three *CBFs* in *Arabidopsis*. These results suggest that *CBF1* or *CBF4* are responsible for the cold response in the *Solanum* species.

Transformants of *S. tuberosum* and *S. commersonii* with the *CBF1* (*ScCBF1*) gene from *S. commersonii* driven by the *35S* promoter were generated and evaluated for drought tolerance in vitro (Pino *et al*. 2013). Both transformants indicated better overall plant growth and higher accumulation of proline compared to NTs. The expression level of *DNH10* was observed to increase during drought stress. This work did not focus on cold tolerance or cold acclimation. Although it is difficult to judge from the information presented here, simple over-expression may not be able to confer the cold acclimation trait to *S. tuberosum*. Further accumulation of studies on cold acclimation using GE techniques is necessary to clarify this mechanism and to confer cold acclimation to *S. tuberosum*.

Future breeding for abiotic stress tolerance

Breeding programs have brought an eagerness to confer biotic stress tolerance to potato and to improve productivity and quality of tubers rather than to enhance abiotic stress tolerance. The majority of recent cultivars and breeding materials in potato carry the above traits. For this reason, it is thought that recent genetic resources in potato may be unfit for breeding abiotic stress tolerance. However, ‘Konyu1~4’ cultivars were established in 2007 by crossing ‘Danshaku’ (Irish Cobbler) and ‘Konafubuki’ (Iwama 2008), whose parental cultivars are major varieties in Japan. ‘Konyu’ showed drought tolerance and had a larger roots mass in comparison to the parent line. Although the practicability of

'Konyu' as a commercial cultivar is unknown, this variety indicates the potential to be a genetic donor for abiotic stress tolerance in recent genetic resources.

On the other hand, *S. tuberosum* has various relatives, including wild species that can exchange genetic traits. Various related species have been utilized as genetic donors to carry out introgression of biotic stress resistance to *S. tuberosum* in the potato pedigree database (Berloo *et al*. 2007). For example cv. 'Astarte' has the cyst nematode resistance gene from *S. vernei*. Using related species as genetic donors to confer abiotic stress tolerance has also been attempted. For example, introgression of traits from *S. commersonii* to *S. tuberosum* has been conducted. However, freezing tolerance and cold acclimation have not transferred into *S. tuberosum* from *S. commersonii* using traditional breeding efforts without the addition of agronomically undesirable wild traits (Cardi *et al*. 1993, Estrada 1982, Estrada *et al*. 1993, Ioveve *et al*. 2004, Pavek and Corsini 2001). In fact, breeding potato cultivars using conventional methods is demanding and time consuming work since *S. tuberosum* is autotetraploid and has a highly complicated quantitative inheritance pattern. Some scientists have suggested that GE represents a faster and a more reliable way to improve potato cultivars (Bayat *et al*. 2010, Waterer *et al*. 2010). However, molecular breeding using GE techniques is not an easy way to establish new abiotic stress tolerance cultivars. In some cases, inconvenient phenotypes appear by conferring the desirable traits mentioned above. Typical examples are transformants with abiotic stress related TFs driven by the *35S* promoter. During controlled conditions, such as in vitro or in growth chambers, inconvenient phenotypes are often hidden (Behnam *et al*. 2006, 2007). These phenotypes gradually become significant in uncontrolled conditions or during large scale cultivation, such as in greenhouses (Shimazaki *et al*. unpublished) and fields. Although the practicability of using transformants with abiotic stress tolerance should be evaluated in the field with some repetition, studies that use continuous field evaluation are still limited. Only one study performed a four-year evaluation in the field (Waterer *et al*. 2010), and they found that conferred traits disappeared in the wet and cool weather conditions. Furthermore, it is difficult to evaluate whether the tolerance levels under controlled conditions, such as in vitro, will reach practical levels in the field. Although similar cases should be applied, compatibility may depend on the transgene, host plant properties and the field trial conditions. For this reason, various case studies, including those that fail, should be collated for future assessment of transgenic potato with abiotic stress tolerance. From the studies introduced above, some transgenes have been shown to enhance abiotic stress tolerance without negative effects. It is expected that these transformants will be planted in fields to provide further information on their performance and tolerance.

In contrast, many wild relative species have thrived in their habitats under various levels of drought conditions. Drought tolerant genotypes were selected from various wild species using seedling growth (Watanabe *et al*. 2011) as the growth profiles differed for each genotype. Although one genotype indicated a slow growth profile in control conditions, seedling growth was not affected under drought conditions (**Fig. 1**). Slow growth is not good as a commercial trait, but this tolerant trait may contribute to potato breeding depending on the mating profile. To accomplish introgression of traits in relative species to *S. tuberosum*, donor plants derived from certain wild relative species have been prepared for future breeding. In the case of conferring abiotic stress tolerance via introgression of their traits, molecular markers are strong tools for screening objective genotypes.

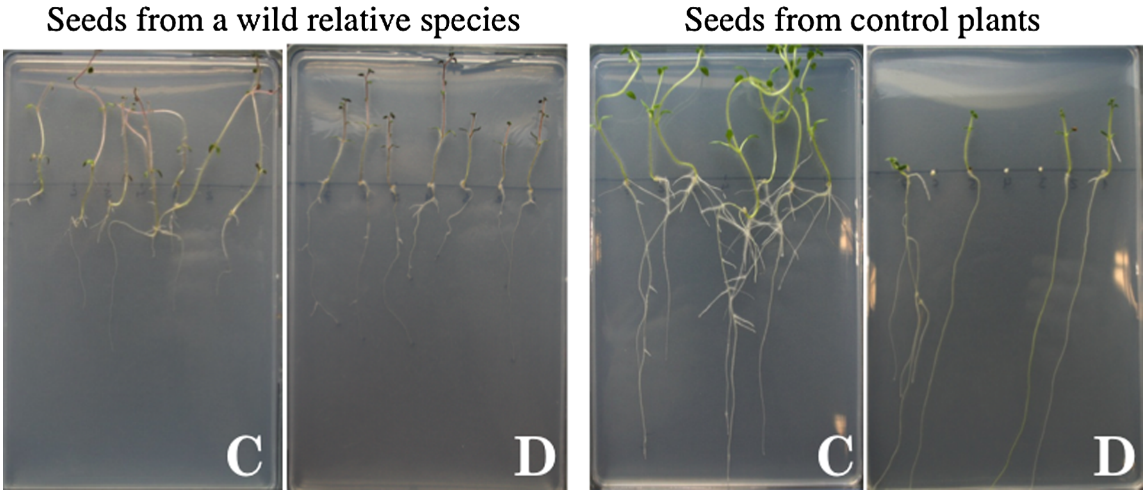

Fig. 1. Seedling growth under drought conditions. Seeds were placed on 1/2 MS solid medium, and cultured at 25°C for 35 days (16L/8D). C indicates control conditions; D indicates drought stress conditions with 4% mannitol. All control plants grew well in control conditions, while their growth was inhibited under drought conditions. Three seeds did not germinate under drought conditions. All plants of the wild species germinated in both conditions, but wild plants grew slower than control plants. However, wild seedlings exhibited better growth under drought conditions than control seedlings.

Table 1. Details of recent transgenic potato studies on abiotic stress referred to in this manuscript

	Host cultivar	Transgene	Origin	Promoter	Treatment*	Plant type**	Evaluation***	Reference
Drought	Desiree	*anti-FDA*	Potato	*35S*	DA	PG	PC	Ambard-Bretteville *et al.* 2003
	Jowon	*YUCCA6*	*Arabidopsis*	*35S*	WW	PG	Sur	Kim *et al.* 2013
	White Lady	*TPS1*	Yeast	*DS2*	Nor	PG	Tr	Kondrak *et al.* 2011
	Gannongshu2	*BADH*	Spinach	*rd29A*	0.5 M Na/15% PEG	Inv	G	Zhang *et al.* 2011
	Superior	*codA*	Rhizobacterium	*SWPA2*	5 μM MV+0.4% Sol/WW/0.15 M Na	SC/PG/SC	G/G/G	Ahmad *et al.* 2008
	Albatros	*GgpPS*	Rhizobacterium	*rd29A/35S*	WD/0.1 M Na	PG	G	Sievers *et al.* 2013
	Desiree	*PR-10a*	Potato	*MAN*	0.5 M Sol or 0.32 M Na	Inv (callus)	G	El-Banna *et al.* 2010
	Superior	*codA*	Rhizobacterium	*SWPA2*	20% PEG	SC	MDA	Cheng *et al.* 2013a
	Desiree	*SST+FFT*	Globe artichoke	*35S*	WD	SC	TP	Knipp and Honermeier 2006
	White Lady	*TPS1*	Yeast	*DS2*	WD	PG	Tr	Kondrak *et al.* 2012
	White Lady	*TPS1*	Yeast	*DS2*	WW	SC	RWC/CF	Stiller *et al.* 2008
Salinity	Jubilei Zhukova	*NHX2*	Barley	*35S*	0~0.25 M Na	Inv	G	Bayat *et al.* 2010
	Gannong2	*NHX1*	*Arabidopsis*	*35S*	0~9 g/L Na	PG	Sur, Y	Wang *et al.* 2010
	Nicola	*P5CS*	*Arabidopsis*	*35S*	0~0.18 M Na	Inv	Y	Hmida-Sayaria *et al.* 2005
	Daejima	*GDP*	Oyster mushroom	*35S*	2 M Na	SC	Sur	Jeong *et al.* 2001
	Marfona	*mtlD*	*E. coli*	*35S*	0~0.15 M Na	H	G	Rahnama *et al.* 2011
Low temperature	Russet Burbank	*AFP*	Synthetic	*35S*	–2.5°C	PG	IL	Wallis *et al.* 1997
	Desnitsa	*Δ12*	Cyanobacterium	*35S*	–7°C /8°C	Inv	IL/Sur,VD	Amiri *et al.* 2010
	Desnitsa	*Δ12*	Cyanobacterium	*35S*	–9°C	Inv	IL, MDA	Demin *et al.* 2008
	Desiree	*ω9*	Wild potato	*35S*	–7~0°C	SC	IL	De Palma *et al.* 2008
	Desiree	*INVase*	Yeast	*B33*	–9°C	Inv	MDA	Deryabin *et al.* 2003
	Desnitsa	*Δ12*	Cyanobacterium	*35S*	–7°C	Inv	MDA	Amiri *et al.* 2007
Oxidative Scavenger	Taedong Valley	*GalURase*	Strowberry	*35S*	10 μM MV/0.1 M Na/0.02 M Zn	Inv	Y	Hemavathi *et al.* 2011
	Atlantic	*SOD+APX+NDPK2*	Cassava, Pea, *Arabidopsis*	*SWPA2*	0~400 μM MV/42°C	SC	D	Kim *et al.* 2010
	Atlantic	*2-cys Prx*	*Arabidopsis*	*SWPA2/35S*	0~300 μM MV/42°C	SC	VD/CF	Kim *et al.* 2011
	Atlantic	*SOD+APX*	Cassava/Pea	*SWPA2*	0~250 μM MV/42°C	SC	VD/RWC	Tang *et al.* 2006
	Atlantic	*NDPK2*	*Arabidopsis*	*SWPA2/35S*	0~250 μM MV/42°C/0.08 M Na	PG	VD/RWC/G	Tang *et al.* 2008
	Desiree	*SOD/DHN4/DREB1/ROB5*	Wheat/Barley/Canola/Bromegrass	*35S/COR78*	44°C/WD/10°C, N	PG, F	G, Yield	Waterer *et al.* 2010
	Superior	*SOD+APX+codA*	Cassava, Pea, Rhizobacterium	*SWPA2*	10 μM MV/0.2 M Na/WW	SC	IL/G/G	Ahmad *et al.* 2010
	Kufri Sutlej	*SOD*	Himalayan cinquefoil	*35S*	WW	SC	PR	Pal *et al.* 2013
	Taedong Valley	*GalURase*	Strowberry	*35S*	0.2 M Na	Inv	G	Upadhyaya *et al.* 2011
	Taedong Valley	*GalURase*	Strowberry	*35S*	0~0.5 M Na	PG	CF	Venkatesh *et al.* 2012
	Taedong Valley	*anti-MSP*	Potato	*35S*	Nor	PG	Y	Gururani *et al.* 2012
	Atlantic	*GR1*	*Arabidopsis*	*35S*	3 μM MV/200 μM Cd, WW	SC/Inv, SC	IL/G,VD	Eltayeb *et al.* 2010
	Atlantic	*DHAR1*	*Arabidopsis*	*35S*	3 μM MV/0.05 M Na/5% PEG	Inv	IL/G/G	Eltayeb *et al.* 2011
	Taedong Valley	*anti-MSP*	Potato	*35S*	0.2 M Na/0.02 M Zn/0.20 M Man	PG	VD	Gururani *et al.* 2013
	Taedong Valley	*GalURase*	Strowberry	*35S*	0~0.2 M Na	Inv	G	Hemavathi *et al.* 2009
	Taedong Valley	*GLOase*	Rat	*35S*	0.1 M Na	Inv	G	Hemavathi *et al.* 2010
Transcriptional factor	Umatilla	*CBF1-3*	*Arabidopsis*	*rd29A/35S*	–7~0°C	SC	IL	Pino *et al.* 2007
	Superior	*EREBP1*	Potato	*35S*	0.075 M Na/8~10°C	Inv	G/Y	Lee *et al.* 2007
	Atlantic	*PF1*	Pepper	*35S*	–9~0°C/45°C/0.4 M/0.075M MV	Inv	IL/Sur/Sur/CF	Youm *et al.* 2008
	Superior	*MYB1R-1*	Potato	*35S*	WW	SC	VD/G/Sur	Shin *et al.* 2011
	LongShu3	*MYB1*	Sweetpotato	*SWPA2*	0.4 M Na/WW	SC	VD	Cheng *et al.* 2013b
	Umatilla	*CBF1*	*Arabidopsis*	*35S*	–16~0°C	SC	IL	Pino *et al.* 2008
	Cardinal	*CBF1*	Wild potato	*35S*	PEG	Inv	G/Sur	Pino *et al.* 2013
	Desiree	*DREB1A*	*Arabidopsis*	*rd29A*	1M Na	Inv	VD	Behnam *et al.* 2006
	Desiree	*DREB1A*	*Arabidopsis*	*rd29A*	2M Na	SC	VD	Celebi-Toprak *et al.* 2005
	Desiree	*DREB1A*	*Arabidopsis*	*rd29A*	–20°C	Inv	Sur	Behnam *et al.* 2007
	Desiree	*DREB1A*	*Arabidopsis*	*rd29A*	PEG	Inv	VD	Huynh *et al.* 2014
	Belle de Fontenay	*DREB1*	Potato	*35S*	0.1M Na/WW	PG	G	Bouaziz *et al.* 2013
	Desiree	*DREB1B*	*Arabidopsis*	*35S*	WW/–6°C	SC	RWC/Sur	Movahedi *et al.* 2012
	Spunta	*DREB2*	Potato	*35S*	0.2 M Na	Inv	G	Bouaziz *et al.* 2012
	Umatilla/*S. commersonii*	*CBF1*	*Arabidopsis*	*35S*	Nor	SC	Tr	Carvallo *et al.* 2011

Plant performance, such as yield or survival rate, and evaluation in near natural conditions are weighed heavily in this list.

* Treatment abbreviations are as follows: Cd: $CdCl_2$, DA: Dry air, Man: Mannitol, MV: Methyl viologen, Nor: Normal conditions, Na: NaCl, PEG: Polyethylene glycol, Sol: Solbitol, WD: Water deficient, WW: Water withheld, Zn: $ZnCl_2$.

** Plant type abbreviations are as follows: F: Field plant, H: Plant in hydroponic culture, Inv: in vitro plant, PG: Potted plant in greenhouse or outside, SC: Plant in soil in controlled conditions.

*** Evaluation abbreviations are as follows: CF: Chlorophyll fluorescence, G: Growth, IL: Ion leakage, MDA: Malondialdehyde content, PC: Proline content, PR: Photosynthetic rate, RWC: Relative water content, Sur: Survival rate, TP: Turgor potential, Tr: Transcriptome, VD: Visible damage, Y: Yield.

Specific makers can be used to retain the objective gene and to eliminate agronomically undesirable wild traits. Studies on transgenic potato with TFs may lead to the development of a specific maker set. It is expected that over-expression of TFs will contribute to the understanding of the mechanisms involved in abiotic stress tolerance and to identify genes directly responsible in potato or wild relatives. The whole genome sequence of *S. phureja* has already been identified (The Potato Genome Sequencing Consortium 2011). Quantitative trait locus (QTL) analysis has become a good method to identify responsible genes by generating transformants of the corresponding DNA region. Although there are many available genes from other plant species, exploring new genes from *Solanum* species is important for the potato breeding program and incorporating abiotic stress tolerance. Conventional breeding and molecular methods, including exploration of responsible genes and maker development using GE techniques, should be used together to develop cultivars tolerant of various abiotic stresses.

Finally, we would like to mention some miscellaneous impressions. Descriptions such as ‘T1 generation’ and ‘wild type (WT)’ were observed in some literatures. This ‘generation’ increases by passing through a tuber, not by mating. Furthermore, ‘WT’ was used to mean the same as a non-transformed host cultivar. Potato is a vegetative propagated cultivar, but not is wild species. These terminologies may be common in some scientists, but we feel a sense of incongruity towards it.

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