Extreme salinity as a challenge to grow potatoes under Mars-like soil conditions: targeting promising genotypes

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Abstract: One of the future challenges to produce food in a Mars environment will be the optimization of resources through the potential use of the Martian substratum for growing crops as a part of bioregenerative food systems. In vitro plantlets from 65 potato genotypes were rooted in peat-pellets substratum and transplanted in pots filled with Mars-like soil from La Joya desert in Southern Peru. The Mars-like soil was characterized by extreme salinity (an electric conductivity of 19.3 and 52.6 dS m⁻¹ under 1 : 1 and saturation extract of the soil solution, respectively) and plants grown in it were under sub-optimum physiological status indicated by average maximum stomatal conductance <50 mmol H₂O m⁻² s⁻¹ even after irrigation. 40% of the genotypes survived and yielded (0.3–5.2 g tuber plant⁻¹) where CIP.397099.4, CIP.396311.1 and CIP.390478.9 were targeted as promising materials with 9.3, 8.9 and 5.8% of fresh tuber yield in relation to the control conditions. A combination of appropriate genotypes and soil management will be crucial to withstand extreme salinity, a problem also important in agriculture on Earth that requires more detailed follow-up studies.

Key words: greenness, Mars soil analogous, salinity, Solanum spp., stomatal conductance.

Introduction

National Aeronautics and Space Administration (NASA) has invested considerable resources (crops identification, growth chambers design, food processing equipment, among others) to guarantee fresh crops growth through bioregenerative food systems (BFS) for future missions to Mars (Perchonok et al. 2012). Although, BFS were mainly focused on artificial growing media (hydroponics, aeroponics, zeoponics, membrane systems), soil-based agriculture (SBA, i.e. using real soil growing media) has become increasingly relevant, achieving even higher productivity in some crops (Nelson et al. 2008). Some authors (Silverstone et al. 2003; Kanazawa et al. 2008; Maggi & Pallud 2010) have pointed out that SBA using in-situ available resource of Martian surface is an important way to guarantee long-term sustainability for the future Martian colony. Mars today is a cold, dry desert world with surface conditions that are not habitable for even the hardest known life forms from Earth (Davila et al. 2010; McKay 2010), however, there is evidence of past (or may be present) water activity and the presence of interesting niches for life (e.g., such as subsurface and/or evaporitic minerals) (Pottier et al. 2017). Moreover, the Martian regolith is very salty and contains exot-ic salts such as sulphates and perchlorates (Hecht et al. 2009) becomes a major challenge for its use in agriculture (Wamelink et al. 2014). In this context, the use of terrestrial analogues of Martian surface constitutes an important effort to know and solve limitations to get SBA in the future (e.g. Silverstone et al. 2003, 2005; Kanazawa et al. 2008; Nelson et al. 2008). Mars-like soils on Earth provide a better understanding the physical, geochemical and microbiological processes that occur, or could have occurred, on Mars (Peters et al. 2008; Valdivia-Silva et al. 2016). Appropriate soil’s analogues on Earth are identified by their similar composition or environmental conditions that describe mechanisms that might guide the search for fossil and living evidence of microbial life (Preston & Dartnell 2014) or/and simulate future problems if Martian soil will be used as a source of future crops and materials for human colonies (Bohle et al. 2016). An interesting Martian soil analogue studied and identified as a key analogue model for life in dry Mars-like conditions.
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is Pampas de La Joya Desert located in southern Peru (Valdivia-Silva et al. 2011, 2016). The very low levels of organic carbon (10–40 ppm) and the presence of exotic minerals (including salts) and oxidants, could allow to identify and analyse the limits of growth in extreme conditions of different plants.

Potato is an extremely versatile crop with thousands of existing varieties adapted to grow well above the Arctic Circle to those able to grow in tropical regions, from 0 up to more than 4000 m above sea level including habitats with extreme weather and soil conditions (Zimmerer 1998; Birch et al. 2012). Wild relatives are found in even more extreme habitats, including extremely arid, saline and frost prone areas and can serve as a source of genetic traits for further adaptation (Martínez et al. 2001; Schäfertner et al. 2007; Vasquez-Robinet et al. 2008; Monneveux et al. 2013). Potatoes are also extremely productive per unit of land area and water usage in comparison with most other staple crops (Renault & Wallender 2000) and are nutritious, rich in digestible starch, protein, fibres, vitamin C and B6, K, Mg and Fe (Woolfe 1986). Therefore, the potato has been considered as a promising crop for growing in space exploration by NASA for many years (Perchonok et al. 2012; Wheeler 2017). An advanced population with wide genetic diversity and stable performance across divergent environments of the subtropical lowland Andigena, resistance to main potato biotic and abiotic stresses, has been developed by International Potato Center (CIP) breeding program (CIP 2017). Such improved materials may prove their value beyond our planet to enable plant production in extreme environments of other planets. In this paper, it is reported a preliminary study testing a large and diverse panel of potato materials including native and improved varieties for their ability to grow and produce tubers in a Mars soil analog from La Joya desert in Southern Peru. The study aims were: to analyse the limiting conditions imposed by the assessed soil – to identify potential materials with higher yield under the tested soil.

Materials and methods

Plant material

Sixty-five genotypes consisting of 38 advanced clones from the CIP Breeding Program for adaptation to subtropical lowlands and tolerance to abiotic stress, 22 native varieties from the taxonomic group Andigena, previously selected for drought tolerance (Cabello et al. 2012) and five improved varieties (see Table 1) were chosen for this experiment. On 30 May 2016 six in vitro plantlets per genotype were transplanted topeat pellets (Jiffy Products Ltd., Canada), which were kept hydrated for 15 days until roots were well developed and plants reached 10–15 cm high.

Soil sampling and characterization

The soil substrate was collected on 2 April 2016 from the hyper-arid area of Pampas de la Joya desert (quadrangle located between 16°38.386‘ S–72°2.679‘ W and 16°44.986‘ S–71°58.279‘ W), extensively studied for its geochemical and Martian characteristics (Valdivia-Silva et al. 2011, 2012, 2016). This desert is the northern part of the Atacama Desert and is located to 50 km of the Arequipa city in Peru. To cover the spatial variability, approximately 700 kg of Mars-like soil was sampled from different points of the desert. The sampled soil was transported to CIP ‘La Molina’ experimental station located in Lima, Peru (12.08° S, 76.95° W, 244 m.a.s.l.) and a composite sample was analysed at Laboratorio de Suelo, Plantas, Aguas y Fertilizantes belonging to Universidad Agraria La Molina, Lima, Peru. The soil was loamy sand (72, 22 and 6% of sand, lime and clay, respectively) with very low organic matter (0.32%) and neutral pH (6.9 and 6.7 under 1 : 1 and saturation extract of the soil solution, respectively). The soil was hyper-saline (an electric conductivity of 19.3 and 52.6 dS m⁻¹ under 1 : 1 and saturation extract of the soil solution, respectively) with a large prominence of Cl⁻¹, Na⁺ and Mg²⁺ (580, 403.4 and 198.4 meq l⁻¹, respectively) as soluble anions and cations.

Experimental conditions and management

On 27 June 2016 six peat-pellets with in vitro plants of each genotype were transplanted in 1 l pots filled with Mars-like soil or a peat-based substrate (PRO-MIX, Premier Tech Horticulture, Canada), the latter serving as a control. All the pots were distributed in six plots randomly distributed in a greenhouse. Every plot had one plant of each genotype: three plots with a plant under Mars-like soil treatment and three plots with a plant under the control condition. All the pots were watered twice per week, to avoid soil leaching and therefore a fully assess for salt tolerance, the water quantity supplied was established through measurement of the maximum evapotranspiration per treatment through the gravimetric method every 2 weeks. For these ten randomly selected individuals per treatment were weighed before the irrigation and the target water quantity per soil treatment was defined as the maximum value estimated to recover the field capacity (see details of this method in Rolando et al. 2015). Based on soil analyses (see the previous sub-section) the fertilizer applications consisted of 200 : 100 : 240 : 20 mg kg⁻¹ as N:P₂O₅ : K₂O : CaO. In total, each pot was fertilized with 37.7 mg of Ca(NO₃)₂, 80.6 mg of NH₄H₂PO₄, 159 mg of NH₄NO₃ and 266.6 mg of KNO₃, distributed in 2, 4, 8 and 6 weekly applications.

The trial duration was 134 days, under this period the average maximum and minimum daily temperature was 19.4 ± 0.2 and 15.2 ± 0.1 °C respectively and atmospheric humidity varied between 94.7 ± 0.3 and 72.0 ± 0.9% (atmospheric temperature and humidity sensor HC283 model, Campbell, USA). The daily average photosynthetic active radiation (PAR, 400–700 nm) was 2.60 ± 0.26, 3.05 ± 0.23, 4.78 ± 0.34 and 5.38 ± 0.29 MJ m⁻² d⁻¹ during July, August, September and October 2016, respectively (LI190SB model, LI-COR, USA). The daily global average atmospheric pressure was 984.3 ± 0.9 mb during July–October 2016 (PTB110 model, VAISALA, Finland).

Plant measurements

Physiological performance of plants under Mars-like condition in relation to the control was assessed through the mid-
morning (taken from 8 to 10 am) or maximum light saturated (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance (\(g_{s,max}\); see details in Ramírez et al. 2016) after and before two water pulses. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance. For this purpose, four genotypes were chosen based on following criteria: (i) contrasted leaf chlorophyll concentration values in relation to the control plants (see formula (1)) assuming that plants with greener leaves in relation to the control were more affected by stress condition imposed by Mars-like soil (see Rolando et al. 2015); and (ii) plants with appropriate leaf size to be assessed in the cuvette (fixing 1200 µmol m\(^{-1}\) s\(^{-1}\) of PAR) stomatal conductance.
Statistical analyses

Two-way ANOVA was performed to assess differences among genotypes, soil treatments and their interaction in fresh tuber yield. A linear regression between ChlSPAD_Amp and % yield was analysed in the surviving genotypes and the most influential points i.e. outliers with significantly affected in the regression line slope – were flagged using Cook’s D and DFFITS tests (Rawlings 1988). All the statistical analyses were run using R software (v. 3.3.3, R Core Team 2017).

Results

The selected genotypes for gs_max assessments showed ChlSPAD_Amp values of 9.3, 14.7, 15.1 and 19.9 corresponding to CIP 304350.18, CIP 388615.22, CIP 309043.123 and CIP 309068.7, respectively. Plants grown in control soil increased their gs_max to 82.7 ± 7.2 and 80.5 ± 17.1% on average after the first and second watering, respectively (Fig. 1). Potatoes in control soil, in particular after water pulses, showed gs_max > 150 mmol H₂O m⁻² s⁻¹, whereas plants growing under Mars-like soil, showed gs_max < 50 mmol H₂O m⁻² s⁻¹ (Fig. 1).

Forty percent of the assessed genotypes survived under Mars-like soil condition with a fresh tuber yield ranging between 0.3 and 5.2 g plant⁻¹ (Fig. 2(a)). The 2-way ANOVA detected significant differences in soil types (F = 541.0, P = 0.048), genotypes (F = 3.9, P < 0.001) and their interaction (F = 4.7, P = 0.031). The % yield as compared with control soil was ranged between 0.3 and 9.3%, being CIP 397099.4, CIP 396311.1 and ‘Tacna’ variety (CIP 390478.9) the genotypes with the highest values (9.3, 8.9 and 5.8%, respectively; Fig. 2(b)). The fitted linear function between ChlSPAD_Amp versus % yield showed a negative slope (y = 19.3 – 1.1x; R² = 0.25) (Fig. 3). The more influent points were the ordinate pairs [x;y]: [1.6%;28.3], [8.9%;5.3] and [9.3%;10.7] defined by Cook’s D (0.17, 0.24 and 0.09, respectively) and DFFITS (0.65, −0.70 and 0.43, respectively) tests (Fig. 3).

Discussion

Physiological performance and tuber yield under Mars-like soil condition

In particular, after water pulses, potatoes in control soil showed gs_max > 150 mmol H₂O m⁻² s⁻¹ (Fig. 1), which has been identified as an appropriate indicator for optimum irrigation and where plants are under optimum conditions (Flexas et al. 2004). On the other hand, plants growing under Mars-like soil and even after water pulses showed gs_max < 50 mmol H₂O m⁻² s⁻¹, which is defined as a physiological severity threshold in potato (Ramírez et al. 2016) where plants are likely submitted to irreversible physiological (oxidative) damage Medrano et al. (2002). This last result and the low tuber yield in relation to the control (Fig. 2) confirms the difficult growing condition characterized by an extremely high soil salinity (see the section Materials and Methods) far beyond that tested in any other studies (from 2.3 to 16.2 dS m⁻¹ of electric conductivity) looking for salt effect in potato (see Katerji et al. 2000; Shaterian et al. 2005; Nagaz et al. 2007). Salts dominated by sulphates, carbonates, chlorides and nitrates are identified as important likely components of Mars regolith (Clark & Van Hart 1981; Osterloo et al. 2008), so extreme salinity conditions such as Mars regolith pose potential problem to grow crops in for future SBA missions (Silverstone et al. 2003; Ewing et al. 2006). It is necessary to design methods to remove or reduce salinity toxicity (e.g. testing previous leaching treatments) but also improve the fertility level of Martian regolith through the incorporation of organic matter recycled.

Fig. 1. Maximum stomatal conductance at saturating light (gs_max, mmol H₂O m⁻² s⁻¹) assessment after and before irrigation pulses (discontinuous lines) in four genotypes (a: CIP 304350.18, b: CIP 309043.123, c: CIP 309068.7, d: CIP 388615.22) growing under standard (black circles) and Mars-like (open circles) soil conditions.
from solid waste composting activities from the human habitat (Silverstone et al. 2003; Nelson et al. 2008). The use of microorganisms to degrade organic matter (Kanazawa et al. 2008) and process remnant salt components (Matsubara et al. 2017), including nanoparticles for soil remediation (Patra et al. 2016), will be important for a sustainable SBA in Mars. Indeed, Martian regolith has high presence of different types of salts and evaporitic minerals i.e. formed by the evaporation from bodies of water (Vaniman et al. 2004; Ewing et al. 2006) and they have been detected on Mars both in situ and remotely by different monitoring instruments (Wadsworth & Cockell 2017). The controversy about their effects on the habitability of that planet is still under research. Thus, some studies have positive implications showing these minerals as possible electron acceptors by microorganisms capable to provide energy for growth or as powerful antioxidants protecting plants against Mars’ harsh environmental stresses and boost the rate of decomposition of organic matter (Bohle et al. 2016). On the contrary, other studies show the Martian salts as a detrimental condition for life survival (Wadsworth & Cockell 2017). The presence of different living beings in extreme salt condition on Earth such as the halophilic organisms encourage the options to generate future crops and better understand the mechanisms of survival in these conditions.

Despite the extreme salinity, 40% of the genotypes survived (Fig. 2). There is a debate if potato is considered as salt sensitive (Maas & Hoffman 1997; Larcher 2003; Nagaz et al. 2007; Levy et al. 2013) or tolerant (Katerji et al. 2000). However, whatever the classification, models estimated as the slope of % yield reduction versus soil electrical conductivity in previous studies (−5.6, −12 and from −34 to −54%/dS m−1 corresponding to Maas & Hoffman 1997; Katerji et al. 2000 and Nagaz et al. 2007, respectively) predict no tuber yield under the salt levels found in the Mars-like soil used in this study. In contrast to these predictions, there was tuber yield as compared the control soil (0.3–9.3%; Fig. 2(b)) highlighting the potential of the assessed genetic material to produce under extreme saline conditions, meriting further studies.

Promising tolerant genotypes and physiological indicators for extreme salinity
CIP 397099.4 and CIP 396311.1, which are advanced clones belonging to CIP lowland tropic virus resistant breeding population (CIP 2017), were identified as the most tolerant to the Mars-like soil with % yield >8% compared with the control (Fig. 2(b)). CIP 396311.1 is an advanced clone with extreme resistance to PVY an PVX, early maturing and tolerant to heat has shown good yields in sites affected by high soil salinity in Southern Bangladesh (Amoros personal communication). The ‘Tacna’ variety (CIP 390478.9) a genotype also with extreme conditions.
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resistance to PVY and PVX, selected from arid and saline environments of the Southern Peruvian Coast (Zegarra & Fernández 2013) showed a yield >5% compared with the control (Fig. 2(b)). This variety is considered as drought and heat tolerant (CIP 2017) with high yields under water restriction conditions reported in Uzbekistan (Carli et al. 2014) and China (locally named as ‘Jizhangshu 8’; He et al. 2013; Wang et al. 2014). Because some mechanisms of resistance are unspecific to the kind of stressors (Larcher 2003), it is expected that genotypes highly resistant to biotic (virus PVY and PVX) and other abiotic (drought and heat) stresses, could also show tolerance to other unreported stressors like salinity. Drought and salinity tolerance share common physiological mechanism (Chaves et al. 2009), so it is expected that some of the traits selected by phenotyping for drought tolerance could confer resistance to salinity also. This was supported by the inverse relationship found between ChlSPAD_Amp and % yield (Fig. 3) predicted by Rolando et al. (2015) under drought stress. Potatoes leaves under stress reduce their growth, concentrating their chlorophyll in less area and appear greener when they are more sensitive to drought (Ramírez et al. 2014; Rolando et al. 2015). Although the predicting capacity of the fitted function was slight ($R^2 = 0.25$), the more influential points were those that showed the higher and lower ChlSPAD_Amp values (Fig. 3), the latter of which corresponded to the genotypes with higher tolerance to Mars-like soil mentioned above (CIP 397099.4 and CIP 396311.1). Greenness inspection through ChlSPAD_Amp may, therefore, be a worthwhile predictor of high tolerant genotypes under extreme salinity that could be used in future breeding programs.

Conclusion

Extreme soil salinity will be an important stressor to the growth of any plants using Martian soil. Under a controlled/protected environment with pressurized atmosphere, a combination of an appropriate sowing method, tolerant genotypes and soil management will be crucial to achieve yield in such conditions. In this preliminary study, In vitro plantlets of two advance clones (CIP 397099.4 and CIP 396311.1) rooted in peat pellets substrate and transplanted into Mars-like soil under drip irrigation, were able to yield more than 8% of tuber biomass as compared with the control under the highest salinity condition reported in scientific studies for potatoes. More studies are necessary to increase the yield in these genotypes through long-stress memory improvement (see Ramírez et al. 2015), to test appropriate controlled atmospheric conditions and soil treatments to reduce extreme salinity effects with a concomitant increase of water and nutrients availability.

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