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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 H_2O 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.

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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 medias (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 hardiest 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 exotic 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 (Martinez *et al.* 2001; Schafleitner *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 agroecologies, 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 to peat 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 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}, $Na^{1+}$and Mg^{2+} (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 $N{:}P_2O_5{:}K_2O{:}CaO$. In total, each pot was fertilized with 37.7 mg of $Ca(NO_3)_2$, 80.6 mg of $NH_4H_2PO_4$, 159 mg of $NH_4.NO_3$ and 266.6 mg of KNO_3, 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 HC2S3 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-

Table 1. *Advances clones (Adv Clone), improved varieties (Imp Variety) and Native potatoes tested in this study conserved in the International Potato Center (CIP) Gene Bank (see further details in CIP Catalogue, CIP 2017). Lowland tropical virus resistant (LTVR) breeding population. Surviving genotypes showed in Fig. 2 are remarked in grey*

CIP number	Population	Biological status	CIP number	Name	Population	Biological status
CIP302428.20	LTVR	Adv Clone	CIP388615.22		LTVR	Adv Clone
CIP302476.108	LTVR	Adv Clone	CIP392820.1		LTVR	Adv Clone
CIP304350.100	LTVR	Adv Clone	CIP394881.8		LTVR	Adv Clone
CIP304350.118	LTVR	Adv Clone	CIP396311.1		LTVR	Adv Clone
CIP304350.18	LTVR	Adv Clone	CIP397099.4		LTVR	Adv Clone
CIP304350.95	LTVR	Adv Clone	CIP390478.9	Tacna		Imp Variety
CIP304366.46	LTVR	Adv Clone	CIP392797.22	UNICA		Imp Variety
CIP304371.20	LTVR	Adv Clone	CIP397077.16	Alliance		Imp Variety
CIP304371.67	LTVR	Adv Clone	CIP374080.5	Perricholi		Imp Variety
CIP304383.41	LTVR	Adv Clone	CIP380389.1	Canchan-INIA		Imp Variety
CIP304387.17	LTVR	Adv Clone	CIP700234	SA-2563		Native
CIP304394.56	LTVR	Adv Clone	CIP700921	Qonpis		Native
CIP309024.1	LTVR	Adv Clone	CIP701531	Yana Rucunag		Native
CIP309024.114	LTVR	Adv Clone	CIP701997	Sullu		Native
CIP309028.32	LTVR	Adv Clone	CIP702363	Soqo Waqoto		Native
CIP309028.56	LTVR	Adv Clone	CIP703264	Kunturpa Chakin		Native
CIP309035.23	LTVR	Adv Clone	CIP703456	Unknown		Native
CIP309043.123	LTVR	Adv Clone	CIP703462	Unknown		Native
CIP309050.36	LTVR	Adv Clone	CIP703488	Challina		Native
CIP309064.76	LTVR	Adv Clone	CIP703502	Rosita		Native
CIP309066.33	LTVR	Adv Clone	CIP703583	Unknown		Native
CIP309068.4	LTVR	Adv Clone	CIP704058	Leona negra		Native
CIP309068.7	LTVR	Adv Clone	CIP704327	Colour Unkhuña		Native
CIP309076.59	LTVR	Adv Clone	CIP704440	Venancia		Native
CIP309077.116	LTVR	Adv Clone	CIP704591	Yana P'utis		Native
CIP309080.60	LTVR	Adv Clone	CIP705088	Chava Negra		Native
CIP309103.85	LTVR	Adv Clone	CIP705223	Capiro		Native
CIP309112.108	LTVR	Adv Clone	CIP705234	Unknown		Native
CIP309112.98	LTVR	Adv Clone	CIP705336	Calvache		Native
CIP309118.5	LTVR	Adv Clone	CIP705490	Muru Warkatina		Native
CIP309121.6	LTVR	Adv Clone	CIP705739	Renacimiento		Native
CIP309126.64	LTVR	Adv Clone	CIP706724	Puka Allqu		Native
CIP309129.11	LTVR	Adv Clone				

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 of a portable photosynthesis system (LI-6400 TX, LICOR, Nebraska, USA). On 20 July 2016 leaf chlorophyll concentration (Chl_{SPAD}) was assessed using a portable chlorophyll meter (SPAD-502 model, Konica Minolta, Japan), for this experiment four readings were taken of an apical leaflet belonging to a young, expanded and sun-exposed leaf and were averaged per plant. For each genotype Chl_{SPAD} amplitude (Chl_{SPAD_Amp}, proposed as stress tolerance index, Rolando *et al.* 2015) was estimated as follows:

$$Chl_{SPAD\_Amp} = X\ Chl_{SPAD\_MS} - X\ Chl_{SPAD\_c} \tag{1}$$

Where $X\ Chl_{SPAD\_MS}$ and $X\ Chl_{SPAD\_c}$ were the Chl_{SPAD} average value in the three plots under Mars-like and control soil treatments, respectively. Harvests (22 September and 8 November 2016) were performed when stems of plants grown in the control soil were brown and had fallen to the ground i.e. code 690 of senescence following Jefferies & Lawson's (1991) classification. In the first harvest, some early genotypes and those that had already died in the Mars-like soil were sampled, whereas in the second harvest the majority of plants were in code 690 of senescence (i.e. 'stems brown and fallen to the ground'). All the tubers were cleaned and weighted and among the surviving genotypes (established as those that survived and yielded in more than two plots) the percentage of fresh tuber yield (g $plant^{-1}$) in relation to the control (*% yield*) was estimated as follows:

$$\%\ yield = \left(\frac{X\ yield_{\mathrm{MS}}}{X\ yield_{\mathrm{c}}}\right)\% \tag{2}$$

where $X\ yield_{\mathrm{MS}}$ and $X\ yield_{\mathrm{c}}$ were the average values of fresh tuber yield in plots under Mars-like soil and control treatments respectively.

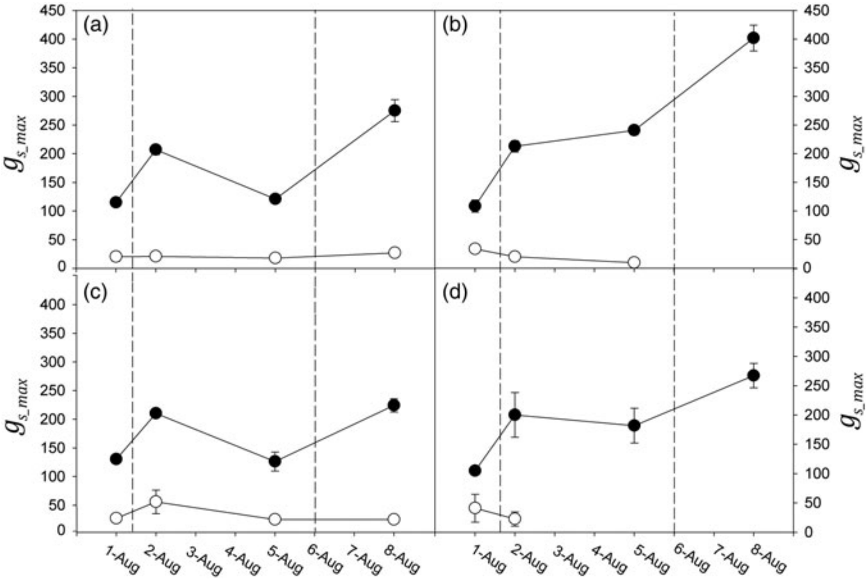

Fig. 1. Maximum stomatal conductance at saturating light (g_{s_max}, mmol H_2O m^{-2} s^{-1}) assessment after and before irrigation pulses (discontinuous lines) in four genotypes (a: CIP 304350.18, b: CIP 309043.123, c: CIP 309068.7, d: CIP 388615.22) growing under standard (black circles) and Mars-like (open circles) soil conditions.

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 Chl_{SPAD_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 g_{s_max} assessments showed Chl_{SPAD_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 g_{s_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 g_{s_max} > 150 mmol H_2O m^{-2} s^{-1}, whereas plants growing under Mars-like soil, showed g_{s_max} < 50 mmol H_2O 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 Chl_{SPAD_Amp} versus *% yield* showed a negative slope ($y = 19.3 - 1.1x$; $R^2 = 0.25$) (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 g_{s_max} > 150 mmol H_2O 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 g_{s_max} < 50 mmol H_2O 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

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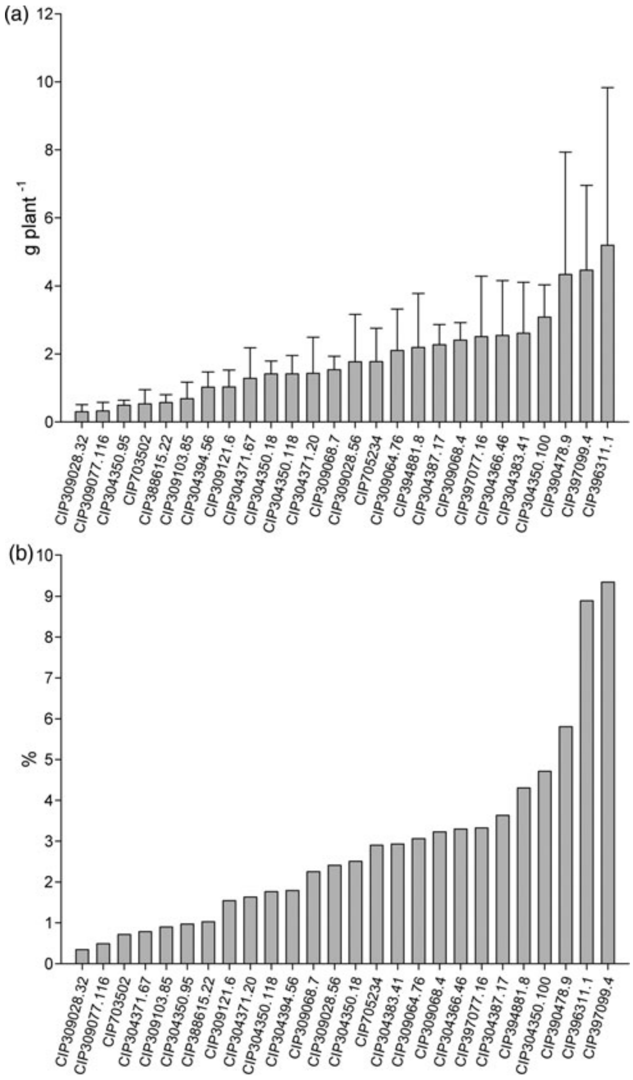

Fig. 2. Fresh tuber yield of the survivor potatoes genotypes growing in Mars-like soil condition expressed as average fresh tuber yield (a) and – average percentage of tuber yield in relation to the yield under the standard soil (b).

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.

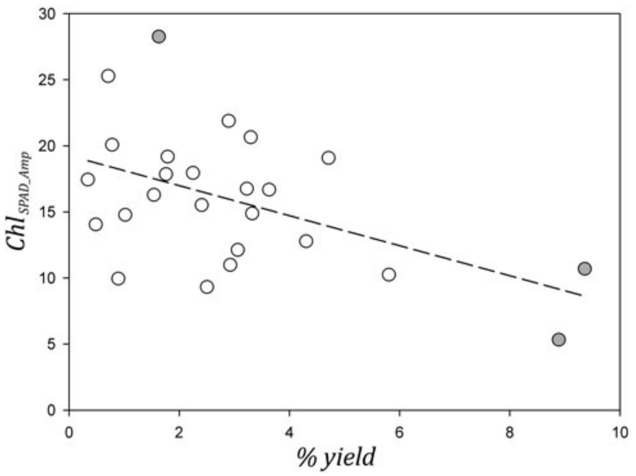

Fig. 3. Scatter plot of the average percentage of fresh tuber yield in potatoes genotypes growing in Mars-like soil in relation to the yield under the standard soil (*% yield*) versus difference of the average chlorophyll SPAD values under Mars-like soil and average chlorophyll SPAD values under the standard soil (Chl_{SPAD_Amp}, without units) measured on 21 July 2016. In grey the more influent points defined by Cook's D and DFFITS tests.

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 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 Chl_{SPAD_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 influent points were those that showed the higher and lower Chl_{SPAD_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 Chl_{SPAD_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 substratum 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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