Evaluation for salt stress tolerance of pepper genotypes to be used as rootstocks

Consuelo Penella [1], Sergio G. Nebauer [2], Salvador Lopéz-Galarza [2], Alberto SanBautista [2], Elisa Gorbe [3] and Angeles Calatayud [1*]

[1]*Instituto Valenciano de Investigaciones Agrarias (IVIA), Departamento de Horticultura Ctra, Moncada-Naquera km. 4,5. 46113-Moncada, Valencia, Spain.* [2]*Universitat Politècnica de València, Departamento de Producción Vegetal, Camino de Vera 14, 46020 Valencia, Spain.* [3]*Wageningen UR Greenhouse Horticulture and Horticultural Supply Chains, Wageningen University and Research Centre, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands. *e-mail: calatayud_ang@gva.es*

Abstract

Salinity is a major environmental constraint on crop productivity and grafting can be a sustainable strategy to enhance plant tolerance under adverse growth conditions. Screening different graft combinations under field conditions can be a slow and expensive processes. In this study, plants of 18 genotypes of *Capsicum* spp. were evaluated during 5 months to select salt tolerant plants to be used as rootstocks in greenhouse under controlled conditions. Their net photosynthetic rate was used as a rapid and sensitive methodology for screening their tolerance to salt stress conditions. The germination potential of some genotypes was also tested under different salinity conditions to see if it would be useful to accelerate the screening process. According to photosynthesis rate, the commercial rootstock 'Tresor' and the genotypes 'Serrano' (*C. annuum*), 'ECU-973' (*C. chinense*) and 'BOL-58' (*C. baccatum*) were the most tolerant during this period. Nevertheless, the evaluation of pepper genotypes for salinity tolerance based on the germination performance and chlorophyll fluorescence parameter Fv/Fm ratio were not good indicators of the sensitivity along plant ontogeny. Finally, the selected genotypes as salt-tolerant were validated under field conditions as rootstocks of two interesting pepper cultivars, concluding that using the rootstocks selected by the net photosynthetic rate improved the salt tolerance of the scion in terms of marketable yield and fruit quality.

Key words: Germination, graft, pepper, photosynthesis, vegetable production.

Introduction

Peppers (*Capsicum* spp.) are economically and socially important crops in the world. Countries in the Mediterranean basin produce around 5,242.450 tn in 234,022 ha. Unfortunately, the continuous soil exploitation, the monoculture and/or intensive agricultural practices have led to the increase of soil salinity [1] and soil-borne diseases, which results in loss of yield and fruit quality [2].

The use of grafted plants is a strategy that allows plants to overcome soil-borne diseases and environmental stresses [3-5]. Their use is becoming more appreciated due to the current trend towards a green and sustainable agriculture. The cultivation of grafted plants has greatly expanded mainly in tomato and watermelon, but this practice is still limited in peppers [4, 6].

Several *Capsicum* rootstocks, including commercial cultivars, breed lines and wild accessions, can give appropriate tolerance or resistance to *Phytophtora, Verticilium, Fusarium,* CMV, etc [7], but there is little information about their tolerance to abiotic stresses.

Abiotic stresses can result in plant senescence, decreasing both yield and product quality [9]. Soil and water salinity are a serious problem in Mediterranean areas where summer crops as peppers are often inevitably irrigated with saline water. Salt tends to accumulate in the soil because of the high evaporative demand is associated to an insufficient leaching ions [10]. In addition, this situation increases the risk of physiological disorders in pepper fruits, particularly in bell peppers, such as blossom-end rot (BER) and cracking. Damages caused by salinity are responsible for high economic losses worldwide [11]. The grafting technique could enable plant breeders to combine desired shoot characteristics with root features that give tolerance to salinity stress [3].

Photosynthesis, together with cell growth, are the primary processes affected by salinity [12, 13]. The effects on photosynthesis performance may be due to stomatal and non-stomatal limitations [14]. The response of photosynthesis to salinity conditions varies depending on plant ontogeny, among different species and also within the same species [15]. Indeed, several studies have demonstrated that the evolution of stress tolerance at various stages of development differs among cultivars of a given species [16]. Therefore, specific stages throughout plant ontogeny, such as germination, vegetative and reproductive stages should be evaluated during the screening period to obtain tolerant plants.

Our aim was to evaluate the behavior of 18 pepper genotypes under salinity conditions in order to select tolerant plants to be used as rootstocks for pepper cultivation. The screening was based on photosynthetic parameters. Furthermore, tolerant

genotypes selected from the screening process were evaluated for their potential of germination under salt conditions. Finally, the selected genotypes were validated as rootstocks of two pepper cultivars in terms of productivity and quality parameters.

Materials and Methods

Experiment 1: Screening pepper genotypes under salinity conditions to be used as rootstocks:
Plant material and growing conditions: The commercial rootstocks cultivars 'Atlante' (Ramiro Arnedo (1)), 'C40' (Ramiro Arnedo (2)), 'Tresor' (Nunhems (3)), the genotypes of *Capsicum annuum* L. 'Serrano Criollo de Morelos' (4), 'Serrano' (5), 'Pasilla Bajío' (6), 'Pimiento de Bola' (7), 'Piquillo de Lodosa' (8), 'Guindilla' (9), and 'Numex Conquistador' (17), the genotypes of *Capsicum chinense* Jacq. 'PI-152225' (11), ECU-973 (12) and 'Morro de vaca' (10), the genotypes of *Capsicum baccatum* L. var. *pendulum* 'BOL-134' (13) and 'BOL-58' (14), the genotypes of *Capsicum pubescens* R.&P. 'BOL 60 amarillo' (15) and 'BOL 60 rojo' (16) and the accession of *Capsicum frutescens* L. 'BOL-144' (18) were used in this study. A numerical code for every cultivar is indicated in brackets. All the genotypes used for the present study belong to the collection of the COMAV institute (Universitat Politécnica de Valencia, Valencia, Spain). Seeds were germinated in moistened perlite at 28 ºC under greenhouse conditions. The seedlings were transferred to 15 L pots containing coconut coir fiber in a heated polyethylene greenhouse on 15th January 2011 in the Instituto Valenciano de Investigaciones Agrarias (Valencia, Spain). Plants were irrigated with Hoagland's No.2 nutrient solution [17].

After 15 days in the pots, plants were divided in two groups for control and saline treatments. Salinity treatment was initiated by adding NaCl (40 mM) to the irrigation solution to reach an EC of 5 dS m^{-1}. The EC of the nutrient solution in the control treatment was 1 dS m^{-1}. Drip irrigation was supplied based on estimations of the weekly crop evapotranspiration (ETc), even though the saline solution was allowed to drain freely from the pots and the control drainage was controlled from 10% to 20% depending on solar radiation.

Eight plants per cultivar were used in each treatment. Plants were grown for 5 months and were kept free from insects and diseases using greenhouse standard management procedures.

Determination of leaf gas exchange and chlorophyll a fluorescence: Net CO_2 fixation rate (A_N, µmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapour (g_s, mol H_2O m^{-2} s^{-1}) and substomatal CO_2 concentration (Ci, µmol CO_2 mol^{-1} (air)) were measured at steady-state conditions under saturating light (1200 µmol m^{-2} s^{-1}) with a LI-6400 (LI-COR, Nebraska, USA). Light curves were previously performed (data not shown) and A_N was saturated at 900 µmol m^{-2} s^{-1}. The CO_2 concentration was 400 ppm and air rate flow 500 µmol s^{-1} .

To evaluate the presence of chronic photoinhibitory processes, the maximum quantum yield of PSII (Fv/Fm = (Fm-Fo)/Fm) was measured on leaves after 30 min of dark adaptation using a portable pulse amplitude modulation fluorometer (MINI PAM, Walz, Effeltrich, Germany). The background fluorescence signal for dark adapted leaves (Fo) was determined with a 0.5 µmol photon m^{-2} s^{-1} measuring light at a frequency of 600 Hz. The application of a saturating flash of 10000 µmol photon m^{-2} s^{-1} enabled estimations of the maximum fluorescence (Fm).

Measurements were performed twice, 2 months (T1) and 5 months (T2) after starting the salinity treatment. During the gas exchange and Fv/Fm measurements the environmental greenhouse ranges were: temperature 21-23ºC, relative humidity 65-70% and solar radiation 800-1200 µmol m^{-2} s^{-1} from 9:00 am to 11:00 am (GMT) in sunny days. Eight plants per treatment were measured on the third or fourth fully expanded leaf from the shoot apex.

Data were analyzed by ANOVA and means were compared using Fisher's least significance difference (LSD) test at p<0.05 (Statgraphics Plus 5.1 for Windows, Statistical Graphics Corp.).

Experiment 2: Potential of germination under salt conditions of the tolerant genotypes selected from experiment 1: From the results of Experiment 1, four genotypes with the highest tolerance to salinity stress (3, 5, 12 and 14) and a sensitive genotype (8) were selected. Seeds of these genotypes were sterilized with 1.5% sodium hypoclorite solution for 7 min, rinsed with sterile distilled water several times, and placed in closed Petri dishes (Ø 9 cm) under aseptic conditions on a Murashige and Skoog culture medium (Sigma-Aldrich) containing 0 (M1), 15 (M2), 40 (M3), 60 (M4) or 100 (M5) mM NaCl for a germination test under salinity conditions. The pH was adjusted to 5.7. Each treatment experiment (genotype x salinity concentration) consisted in four separated replicates of 100 seeds. Seeds were allowed to germinate in a phytotron (Sanyo MLR-350H) at 25ºC, 85% RH and 16 h irradiance (PAR: 45 µmol m^{-2} s^{-1}). The number of germinated seeds was recorded daily using radicle extrusion (≥ 2 mm long) as criterion.

Germination data of each replicate were fitted to the logistic function [18] $G = A[1 \pm exp (\beta - kt)]^{-1}$, defined as a special form of the Richards function, where G = cumulative germination (%), t = germination time (days), and A, β and k are function parameters, being A the maximum germination percentage (asymptote when t→∞), k is a "rate parameter" and β places the curve in relation to the time axis, without any biological significance. In addition, derived quantities with biological significance were also calculated, as the time at the inflexion point to reach 50% of final germination percentage ($Gt\ 50 = \beta/k$, *days*), and mean relative cumulative germination rate ($k/2$, days^{-1}). Variables (A, β/k and $k/2$) were analysed by ANOVA (Statgraphics Plus 5.1 for Windows, Statistical Graphics Corp.). Percentage data were arcsin transformed before analysis. Mean separations were performed with the LSD test at $P<0.05$.

Experiment 3: Grafting of two cultivars onto four selected genotypes of experiment 1: The experiment was performed during 2012 in a sweet pepper producing area in Valencia, Spain, using 'Adige' F1 (Lamuyo type, Sakata Seeds, Japan) and 'Lipari' F1 (Italian type, Clause Spain) cultivars, grafted onto four salinity tolerant genotypes 3, 5, 12 and 14, according to the results obtained in Expt. 1. Ungrafted 'Adige' and 'Lipari' plants were used as controls. Grafted and ungrafted plants were transplanted on the 21th of March at a density of 2.1 plants m^{-2} in a sandy soil (pH 8.0, EC$_{(1/5)}$ 1.2 dS m^{-1}, Sand 76%), under polyethylene greenhouse. The electrical conductivity and pH of the irrigation water were 3.5 dS m^{-1} and 7.60, respectively, with 88 meq l^{-1} of Na$^+$ and 111 meq l^{-1} of Cl$^-$. Fertilizers were applied at a rate (kg ha^{-1}) of 200 N, 50 P$_2$O$_5$, 250 K$_2$O, 110 CaO and 35 MgO [19].

The tube grafting method was used, by cutting the growing tip of the rootstock at a 45º angle below cotyledons, attaching

subsequently the scion, previously cut at a 45º angle above cotyledons and fixing rootstock and scion with a clip.

Harvest was performed from the end of May until the end of July. Fruits were graded in two classes: marketable and unmarketable fruits. The latter fruits were mainly (>95%) affected by BER.

A randomized complete block design was performed with three replicates, each consisting of 25 plants. Data were subjected to ANOVA and means were compared using Fisher's least significant difference (LSD) test at $P<0.05$ (Statgraphics Plus 5.1 for Windows, Statistical Graphics Corp.).

Results

Effect of stress conditions on photosynthetic parameters: Pepper genotypes grown under control conditions in this study differed significantly ($P<0.05$) among themselves in the net CO_2 fixation rate (Fig. 1). The genotypes 2, 4, 6, 7, 8, 10, 13 and 14 showed the highest photosynthetic rates, with values near or above 20 μmol CO_2 m^{-2} s^{-1}. In contrast, genotypes 1, 3, 11 and 12 showed the lowest rates.

In March (T1), after 2 months of salinity conditions, the genotypes 2, 3, 5, 12, 13 and 14 maintained the photosynthetic rate and stomatal conductance under salt conditions, when compared to controls (Fig. 1A- B). In genotypes 6 and 11, stomata closed by the effect of salinity, although A_N remained unaffected. Net photosynthesis rate and stomatal conductance decreased due to salt stress in the remaining genotypes (Fig. 1A-B).

After 5 months of treatment (T2), the net photosynthetic rate in genotypes 1, 3, 5, 12 and 14 did not differ from controls under salinity conditions (Fig. 1C). However, only genotype 1 maintained the stomatal conductance (Fig. 1D) in response to salt conditions without significant differences respect to control. Nevertheless, comparing the g_s value of the 18 genotypes in the control treatment, genotype 1 had the lowest.

Along T1 and T2 a logarithmic correlation between net photosynthetic rate and stomatal conductance was observed ($A_N = 6.62 \ln g_s + 26.4$; $R^2 = 0.80$). In addition, stomatal conductance was also related to substomatal CO_2 concentration ($C_i = 67.6 \ln g_s + 355$; $R^2 = 0.86$).

Fv/Fm ratio did not changed along the experiment neither among genotypes nor treatments (data not show).

In summary, the net photosynthetic rate of the genotypes 'Tresor' (3), 'Serrano' (5), 'ECU-973' (12) and 'BOL-58' (14) was not affected by salinity conditions in both measurements time.

Seed germination test: The selected salt-tolerant genotypes in Experiment 1 (3, 5, 12 and 14) and the genotype 8 (sensitive to salinity) were used to test the germination potential under salinity conditions.

The ANOVA of the logistic function parameters A,β /k and k/2 of our studies are shown in Table 1. The interaction "Genotype x Salinity" was significant ($P<0.01$). The coefficients of determination (R^2) for curves ranged from 0.90 to 0.989 and F ratio values of the statistical model were significant ($P<0.01$). The source of variation ($P<0.01$) for β/k was 79.79 of total sum of square appeared for genotypes.

The fitted curves corresponding to the average values of each variance source (genotype and NaCl concentration) are shown in Fig. 2. High values (>80%) of final germination were reached in
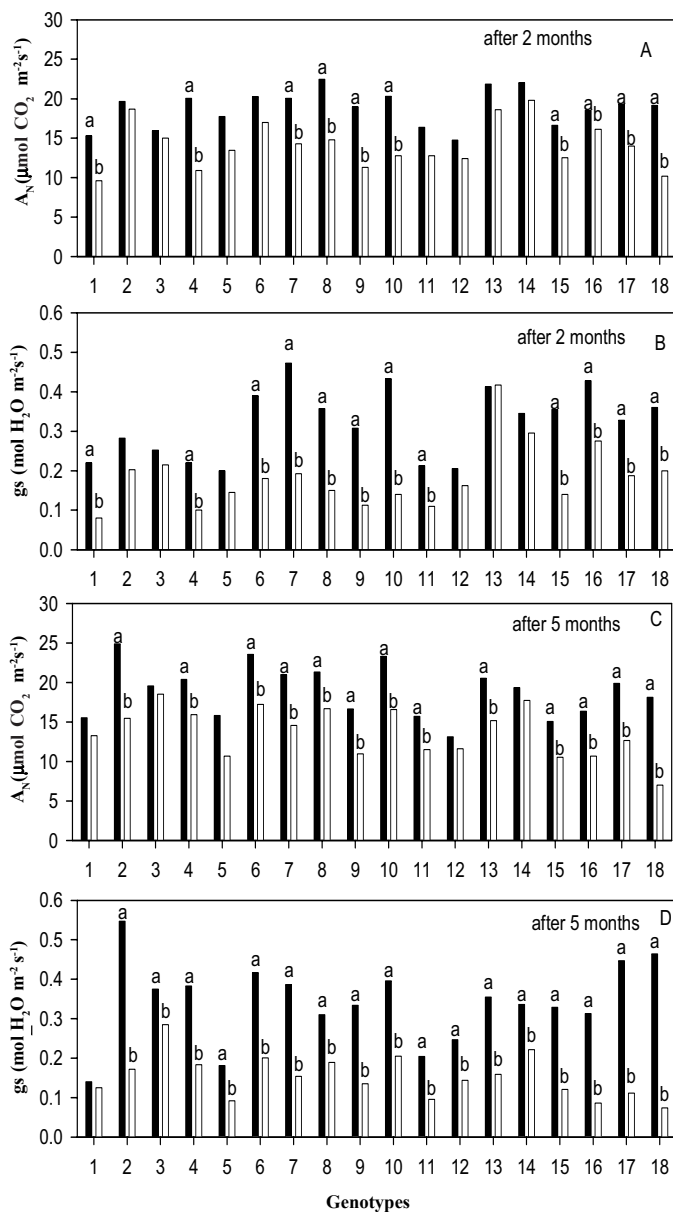

Figure 1. Leaf gas exchange parameters in pepper genotypes measured after 2 (T1) and 5 months (T2) in the control (1 dSm^{-1}, ■) and irrigated with saline water (5 dSm^{-1}, □). A_N net photosyntesis at T1 (A) and T2 (C) and stomatal conductance to water vapour (g_s) at T1 (B) and T2 (D). Values are means of 8 samples. For comparision of means, analysis of variance (ANOVA) followed by the least significance differences (LSD) test calculated at P < 0.05, were performed. Values for each genotype followed by different letter indicate significant differences. Non letter indicates non-significant difference for each genotype.

most of combinations under salinity conditions. The seeds with higher A values had also a higher germination rate. The seeds of the genotypes 8 and 12 showed the slower germination rates, requiring higher periods to reach 50% of final germination. By contrast, the genotype 5 showed the highest values of maximum germination percentages, requiring the shortest periods to reach 50% of final germination (Fig. 2).

Table 1. Analysis of variance of parameters A(%), β/*k* (days) and *k*/2 (days^{-1}), obtained by curve-fitting to logistic function for seeds from different genotypes and salt concentrations.

	Percentage of the total sum of square [+]		
Source	A	β/K	k/2
Genotypes	11.970**	79.798**	48.972**
Salt concentration	19.911**	9.252**	8.918**
Genotypes x Salt	28.612*	6.807**	18.850**
Error	39.507	4.143	23.260
Standard deviation [++]	3.066	0.649	0.761

*,** Indicated significant at pd≤0.05 and pd≤0.01, respectively.
[+] For each component, the sum of squares is given as a percentage of the total.
[++] Calculated as the square root of the nutrient between the absolute value of the residual sum of squares (using the units indicate) and the degrees of freedom of the error.

Field experiment: In general terms and for both cultivars 'Adige' and 'Lipari', genotype 3 and followed by genotype 14, gave the best response when used as rootstocks (Table 2). Furthermore, in all grafted plants the efficiency was higher compared with ungrafted plants (except in genotype 12 grafted onto 'Lipari'). This was reflected by a higher marketable yield and lower unmarketable yield due to BER. However, total yield was similar for 'Adige' in all grafted or ungrafted plants. No differences were found between marketable yield of ungrafted plants and grafted plants onto genotypes 5 and 12.

In 'Lipari', marketable yield was lower when 5 and 12 genotypes were used, mainly for 12. The highest losses in the unmarketable fruits were achieved in ungrafted plants with significant differences respect to grafted plants (Table 2).

The percentage of fruits affected by BER was lower in 'Lipari' than in 'Adige'. The fruit number and the mean fruit weight of commercial fruits were similar in all 'Lipari' combinations but not in 'Adige' plants, where a lower number heavier fruits were obtained when using rootstock 12.

Discussion

The productivity of several commercial pepper crops (mainly bell peppers) is limited by salinity stress in many areas of the world. The screening of salt-tolerant pepper has been developed mainly in genotypes with poor commercial value [20, 21]. A new perspective for screening genotypes is their use as rootstocks to improve the tolerance of a desirable cultivar to abiotic stresses. With this aim we tested 18 pepper genotypes grown under salinity conditions in a greenhouse in the Mediterranean area.

Salinity can affect photosynthesis as a result of ion imbalance, ion toxicity and osmotic stress in plants [14, 22]. A limitation of CO_2 supply due to partial stomatal closure has been described as an early response to salt stress [21]. In this work, stomatal conductance and photosynthesis were negatively affected by salinity in T1 and T2 periods in some genotypes. The low substomatal CO_2 concentration under stomata closure suggested stomatal constraints to photosynthesis. Stomatal conductance decreased in salinity conditions in genotypes 3, 5, 12 and 14 at T2 but not at T1, although A_N did not show significant differences along the experiment in these genotypes when compared to controls. This can be explained by the fact that only very critically low levels of g_s in these genotypes affected photosynthesis, which is in agreement with [5, 23].

The reduction in photosynthesis rate can also be due to alterations in leaf photochemistry [14]. The leaf chlorophyll fluorescence ratio, Fv/Fm is a classic parameter reflecting the whole PSII function, and its decrease is associated with PSII damage or photoinhibition under environmental stresses [24]. In our experiment, the Fv/Fm measured both periods did not show significant differences between control and stress treatments, implying that PSII activity was not affected by salt stress. Other studies have shown little or no effect on Fv/Fm [21, 25-27] even when leaf growth and gas exchange were reduced. The decrease of stomatal conductance and the photosynthesis rate, leaving PSII unaffected suggested a highly resistant PSII activity under stress conditions [26, 28] and/or that the limitation of photosynthesis by reduction of Rubisco activity does not occur until this stress is highly severe [14, 29]. Based on fluorescence and gas exchange parameters, our results suggested that diffusional restriction is the main factor that limits photosynthesis in sensitive pepper genotypes in our salinity conditions.

Since the limitation by CO_2 was the main factor responsible for the decrease in the net photosynthetic carbon uptake rate [30], we selected the A_N as the indicator parameter of sensitivity or tolerance with regard to salinity stress.

Table 2. Production parameters: yield (kg m^{-2}), fruit number per m^2 and mean fruit weight (g fruit^{-1}) of marketable and unmarketable yield, percentage of the number of unmarketable yield (%) and total yield (kg m^{-2}).

Cultivar	Rootstock genotype	Marketable yield			Unmarketable yield			Total yield
		kg m^{-2}	Fruit number m^{-2}	g fruit^{-1}	kg m^{-2}	%	Fruit number m^{-2}	kg m^{-2}
Adige	3	3.4 a	26.3 a	129.5 b	1.5 b	30.3 c	8.5 b	4.9
	5	1.5 bc	15.2 bc	98.8 b	3.6 a	72.2 a	25.8 a	5.1
	12	1.8 bc	10.0 c	187.8 a	2.2 b	56.4 ab	19.8 a	3.9
	14	2.5 ab	19.9 ab	125.8 b	1.9 b	40.1 bc	7.6 b	4.4
	Ungrafted	1.4 c	12.1 bc	112.9 b	3.2 a	70.7 a	17.7 a	4.6
	Significance (F values)	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	NS
Lipari	3	4.1 a	14.3	289.6	1.5 c	26.3 c	2.1 b	5.6 a
	5	3.1 bc	14.3	220.4	1.7 b	36.4 ab	24.3 a	4.8 ab
	12	2.6 c	13.0	206.7	1.3 c	34.0 b	21.2 a	3.9 b
	14	3.9 ab	15.2	257.2	0.8 d	17.0 d	7.2 b	4.7 ab
	Ungrafted	3.1 bc	11.8	267.4	2.1 a	41.3 a	13.5 ab	5.3 a
	Significance (F values)	P<0.05	NS	NS	P<0.01	P<0.01	P<0.05	P<0.05

Values are mean of n=50 plants of cultivar 'Adige' and 'Lipari' grafted or not onto genotype 3, 5, 12 and 14. Different letters in each column indicate significant differences at *P*<0.05 using the LSD test. NS – not significant.
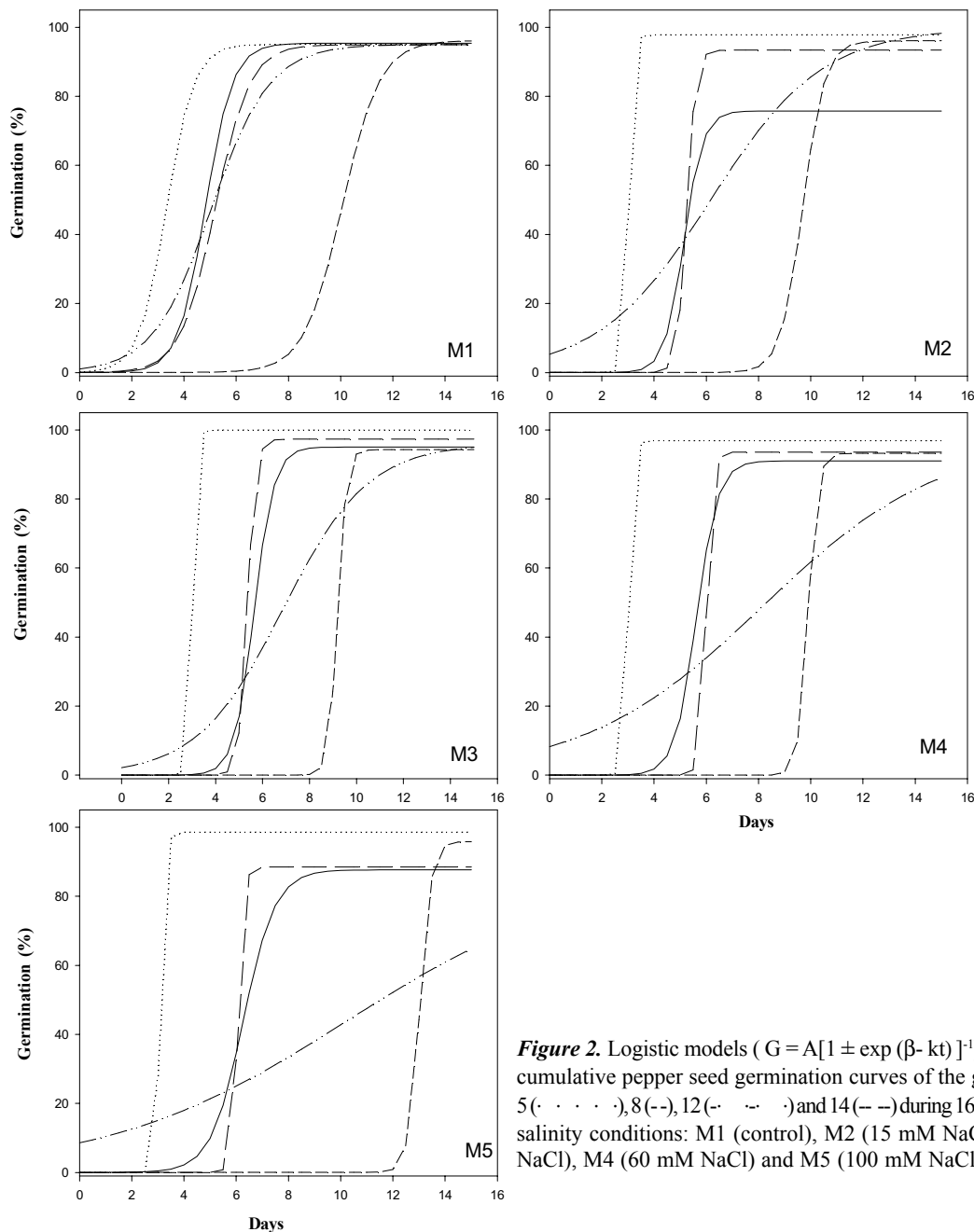
Figure 2. Logistic models ($G = A[1 \pm \exp(\beta - kt)]^{-1}$) fitted to cumulative pepper seed germination curves of the genotypes: 3 (–), 5 (· · · · ·), 8 (- -), 12 (-· -· ·) and 14 (– –) during 16 days under different salinity conditions: M1 (control), M2 (15 mM NaCl), M3 (40 mM NaCl), M4 (60 mM NaCl) and M5 (100 mM NaCl).

We observed some differences in the photosynthetic performance between T1 and T2 in some pepper genotypes. Genotypes 2, 6, 11 and 13 showed a decrease in A_N at T2 but not at T1 under salinity condition, and only genotype 1 did not show significant differences at T2 but it did at T1. These results may indicate that the these genotypes after a period of salinity exposure were more sensitive under our growth conditions. A longer exposure to salinity, which may cause ion accumulation in the leaves [31] or alterations in osmotic adjustment [32] and can be the cause of higher photosynthetic decrease.

Since many crops show different sensitiveness at different stages of their ontogeny, others may have a similar response among them. In that case, determining the response of the seeds in terms of the germination performance under salinity stress conditions would be useful to accelerate the screening process.

The sensitivity or tolerance to salinity during the germination stage is species-dependent; many crops are vulnerable to stress during seed germination [33], while others are relatively tolerant [34]. Salt stress can reduce germination either by limiting water absorption by the seed [35], by affecting the mobilisation of stored reserves [36], by directly affecting the structural organization or synthesis of proteins in germinated embryos [37] or by intake of toxic ions, which may affect metabolic activities [38]. In our study, in general terms, the maximum germination rate decreased and/or the seeds required a longer period to reach the 50% of the final germination percentage as NaCl increased in the media. However the magnitude of this response varied among genotypes. Those that germinated rapidly at low stress conditions also germinated properly at high stress levels. The same effect was demonstrated in different *Lycopersicum* accessions under salinity [33]. The genotype 5 showed the highest germination rate even at 100 mM NaCl and it had higher photosynthetic rate under salinity conditions at T1 and T2 , On the other hand, genotype 12 showed a lower maximum germination rate and required a longer period to

reach the 50% of final germination percentage, but it had a higher photosynthesis rate under salinity stress at T1 and T2. This indicates that this genotype is more sensitive to salinity stress during germination or requires more time to germinate. Finally, genotype 8 took longer time to germinate, even in the control treatment, compared to other genotypes, at the end the germination percentage was high but this cultivar was sensitive to salinity stress at T1 and T2.

The observed differences in the response to salinity in the genotypes during germination phase was not representative of salinity tolerance of these potential rootstocks during T1 and T2. As a consequence, our results indicate that the screening of pepper genotypes for salinity tolerance based only in the germination performance is not a good indicator of their sensitivity in the adult plant-stage. Nevertheless, the selection of a desirable rootstock only for the germination phase is not limiting factor because from an agronomic point of view grafting is done in commercial nurseries, where seeds are germinated in optimal conditions, i.e. no saline water and substrates, due to the high cost of the seeds.

The screening of salt tolerant genotypes has been used for the introduction of salt-tolerant crops [20, 21], although these crops often have poor yield and low quality fruits. Grafting is a well established technique for crop production under salinity conditions [3, 39]. The increase of salt tolerance observed in grafted plant is due to the use of salt tolerant rootstocks, although plants grafted onto different rootstocks respond more or less differently to salinity [40]. Several authors (see review [3]) have reported an increase in growth and fruit yield in grafted plants under salinity conditions, mainly in tomato [40, 41], watermelon [42, 43] or eggplants [44, 45], but there are few studies about the effect of grafting of pepper plants under salinity conditions. Our proposal is the use of selected salt tolerant pepper genotypes as rootstocks. Pepper is classified as moderately sensitive to salt stress but its response is cultivar-dependent [21, 46]. The graft of interesting but salt-sensitive pepper cultivars ('Adige' and 'Lipari') onto our salt tolerant genotypes (3, 5, 12, 14) may provide the capacity for inducing salt tolerance on them. In general terms, our results indicate that the selected tolerant genotypes induced a better response to the scions when used as rootstocks in comparison with ungrafted plants. The grafted pepper plants had, in general higher marketable yields compared with the ungrafted cultivars when cultured in high saline water and soil. The marketable yield with regard to the total fruit yield was relatively low in ungrafted 'Adige' plants (30%) and in 'Adige' plants grafted on genotype 5 (29%) compared with those grafted on 3 (70%), 14 (57%) and 12 (45%). The genotype 5 resulted in lower marketable yields when used as rootstock of both cultivars. This genotype seems to have affinity problems, as the same has been observed in other experiment under water stress conditions [47] and this fact could explain the lower marketable fruit yields.

More experiments need to be carried out in this direction to understand the interactions rootstock-scion in these selected genotypes.

The occurrence of BER was the main cause of the unmarketability of the fruits in both tested cultivars. Pepper has been described as very susceptible to BER [48]. This disorder has been associated to a local deficiency of calcium [49]. The high incidence of BER in our experiment could be due to the high salinity both in the nutrient solution and soil, combined with the climatic conditions

of spring-summer, with high temperatures and high leaf transpiration rates as it has been observed [50] in pepper, and this can diminish the calcium partitioned to the fruits. In our study, tolerant rootstocks to saline stress significantly decreased the percentage of BER in the fruits respect to ungrafted plants, although it was high due to our salinity conditions, this is an important conclusion from this study, as this disorder adversely affect marketable yields and it can diminish using tolerant rootstocks. On the other hand, the occurrence of BER not only depended on the rootstock but also on the scion used since 'Lipari' had lower percentage of BER than 'Adige'. Therefore, the salt tolerance of the grafted plants is a combination of the salt tolerances of both scion and rootstock [39].

Conclusions

The photosynthetic rate A_N is a useful sensitive parameter for the selection of salt tolerant pepper genotypes and it is related to the plant production, which has been validated in terms of yield and fruit quality under salinity conditions. The use of salt-tolerant rootstocks has been proved as an excellent and sustainable strategy to improve the salt tolerance of pepper plants, even though the level of improvement depends on the sensitivity of the scion. Wild pepper genotypes used as rootstocks are interesting as a source of tolerance to salinity stress. Nevertheless, further studies are needed to search the best scion/rootstock combinations in order to optimize the crop value.

Acknowledgements

References
[1]Rozema, J. and Flowers, T. 2008. Crops for a salinized world. Science **322**:1478-1480.

[2]Lacasa, A., Guerrero, M. A., Guirao, P. and Ros, C. 2002. Alternatives to methyl bromide in sweet pepper crops in Spain. In Batchelor, T. *et al.* (eds). Proceedings of the International Conference Alternatives to Methyl Bromide. The Remaining Challenges. European Comission, Brussels, Belgium, pp. 172-177.

[3]Colla, G., Rouphael, Y., Leonardi, C. and Bie, Z. 2010. Role of grafting in vegetable crops grown under saline conditions. Sci. Hortic. **127**:147-155.

[4]King, S. R., Davis, A. R., Zhang, X. and Crosby, K. 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Sci. Hortic. **127**:106-111.

[5]San Bautista, A., Calatayud, A., Nebauer, S. G., Pascual, B., Maroto, J. V. and López-Galarza, S. 2011 Effects of simple and double grafting melon plants on mineral absorption, photosynthesis, biomass and yield. Sci. Hortic **130**:575-580.

[6]Miguel, A., De la Torre, F., Baixauli, C., Maroto, J. V., Jordá, M. C., López, M. M. and García-Jiménez, J. 2007. Injerto de hortalizas. Ministerio de Agricultura, Pesca y Alimentación y Fundación Ruralcaja. Valencia. Spain, 63 p.

[7]Morra, L. 2004. Grafting in vegetable crops. In International Workshop. The Productions in the Greenhouse after the Era of the Methyl Bromide, pp. 147-154.

[8]Kokalis-Burelle, N., Bausher, M. G. and Rosskopf, E. N. 2009. Greenhouse evaluation of Capsicum rootstocks for management of *Meloidogyne incognita* on grafted bell pepper. Nematropica **39**:121-

132.

9López-Marín, J., González, A., Pérez-Alfocea, F., Egea-Gilabert, C. and Fernández, J. A. 2013. Grafting is an efficient alternative to shading screens to alleviate thermal stress in greenhouse-grown sweet pepper. Sci. Hortic.**149**:39-46.

10De Pascale, S., Ruggiero, C., Barbieri, G. and Maggio, A. 2003. Physiological responses of pepper to salinity and drought. J. Am. Soc. Hort. Sci. **128**:48-54.

11Rai, M. K., Kalia, R. K., Singh, R., Gangola, M. P. and Dhawan, A. K. 2011. Developing stress tolerant plants through *in vitro* selection-An overview of recent progress. Env. Exp. Bot. **71**:89-98.

12Munns, R., James, R. A. and Läuchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereal. J. Exp. Bot. **57**:1025-1043.

13Parida, A. K. and Das, A. B. 2005. Salt tolerance and salinity effects on plants: A review. Eco. Environ. Saf. **60**:324-349.

14Chaves, M. M., Flexas, J. and Pinheiro, C. 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Ann. Bot. **103**:551-560.

15Chartzoulakis, K. and Klapaki, G. 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hortic. **86**:247-260.

16Almansouri, M., Kinet, J. M. and Lutts, S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil **231**:243-254.

17Maynard, D. N. and Hochmuth, G. J. 1997. Knott's Handbook for Vegetable Growers. John Wiley & Sons, Inc., New York, 78 p.

18Torres, M. and Frutos, G. 1989. Analysis of germination curves of aged fennel seeds by mathematical-models. Env. Exp. Bot. **29**:409-415.

19Maroto, J. V. 2002. Horticultura Herbácea Especial. 5ª Edición. MundiPrensa, Madrid, Spain, 465 p.

20Aktas, H., Abak, K. and Cakmak, I. 2006. Genotypic variation in the response of pepper to salinity. Sci. Hortic. **110**:260-266.

21Niu, G., Rodriguez, D. S., Crosby, K., Leskovar, D. and Jifon, J. 2010. Rapid screening for relative salt tolerance among Chile pepper genotypes. HortSci. **45**:1192-1195.

22Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. **59**:651-681.

23Flexas, J., Bota, J., Loreto, F., Cornic, G. and Sharkey, T. D. 2004 Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biol. **6**:269-279.

24Baker, N. R. 2008. Chlorophyll fluorescence: A probe of photosynthesis *in vivo*. Annu. Rev. Plant Biol. **59**:89-113.

25Naumann, J. C., Young, D. R. and Anderson, J. E. 2007. Linking leaf chlorophyll fluorescence properties to physiological responses for detection of salt and drought stress in coastal plant species. Physiol. Plant. **131**:422-433.

26Lee, H.Y., Jun, S. S. and Hong, Y. N. 2004. Increased photoinihibition in dehydrated leaves of hot pepper (*Capsicum annuum* L.) is not accompanied by an incremental loss of functional PSII. J. Plant Biol. **47**:83-91.

27Yan, K., Chen, P., Shao, H., Zhao, S., Zhang, L., Xu, G. and Sun J. 2012. Responses of photosynthesis and photosystem II to higher temperature and salt stress in sorghum. J. Agron. Crop Sci. **198**:218-226.

28Baker, N. R. and Rosenqvist, E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. J. Exp. Bot. **55**:1607-1621.

29Bota, J., Medrano, H. and Flexas, J. 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress?. New Phytol. **162**:671-681.

30Chaves, M. M. and Oliveira, M. M. 2004. Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. J. Exp. Bot. **55**:2365-2384.

31Greenway, H. and Munns, R. 1990. Mechanisms of salt tolerance in non-halophytes. An. Rev. Plant Physiol. and Plant Mol. Biol. **31**:149-190.

32Silva, C., Martínez, V. and Carvajal, M. 2008. Osmotic versus toxic effects of NaCl on pepper plants. Biol. Plant. **52**:72-79.

33Foolad, M. R. and Lin, G. Y. 1997. Genetic potencial for salt tolerance during germination in *Lycopersicon* species. HortSci. **32**:296-300.

34Murillo-Amador, B., López-Aguilar R., Kaya, C., Larrinaga-Mayoral, J. and Flores-Hernández, A. 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. J. Agron. Crop Sci. **188**:235-247.

35Dodd, G. L. and Donovan, L. A. 1999. Water potencial and ionic effects on germination and seedling growth of two cold desert shrubs. Am. J. Bot. **86**:1146-1153.

36Bouaziz, A. and Hicks, D. R. 1990. Consumption of wheat seed reserves during germination and early growth as affected by soil-water potential. Plant Soil **128**:161-165.

37Ramagopal, S. 1990. Inhibition of seed-germination by salt and its subsequent effect on embryonic protein-synthesis in barley. J. Plant Physiol. **136**:621-625.

38Smith, P. T. and Comb, B. G. 1991. Physiological and enzymatic activity of pepper seeds (*Capsicum annuum*) during priming. Physiol. Plant. **82**:71-78.

39Huang, Y., Bie, Z., Liu, P., Niu, M., Zhen, A., Liu, Z., Lei, B., Gu, D., Lu, C. and Wang, B. 2013. Reciprocal grafting between cucumber and pumpkin demonstrates the roles of the rootstock in the determination of cucumber salt tolerance and sodium accumulation. Sci. Hortic. **149**:47-54.

40Estañ, M. T., Martinez-Rodriguez, M. M., Perez-Alfocea, F., Flowers, T. J. and Bolarin, M. C. 2005. Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. J. Exp. Bot. **56**:703-712.

41Santa-Cruz, A., Martinez-Rodriguez, M. M., Perez-Alfocea, F., Romero-Aranda, R. and Bolarin, M. C. 2002. The rootstocks effect on the tomato salinity response depends on the shoot genotype. Plant Sci. **162**:825-831.

42Goreta, S., Bucevic-Popovic, V., Selak, G. V., Pavela-Vanric, M. and Perica, S. 2008. Vegetative growth, superoxide dismutase activity and ion concentration of salt-stressed watermelon as influenced by rootstock. J. Agri. Sci. **146**:695-704.

43Yetisir, H. and Uygur, U. 2010. Responses of grafted watermelon onto different gourd species to salinity stress. J. Plant Nutr. **33**:315-327.

44Liu, Z. I., Zhu, Y. L., Wei, G. P., Yang, L. F., Zhang, G. W. and Hu, C. M. 2007. Metabolism of ascorbic acid and glutathione in leaves of grafted eggplant seedling under NaCl stress. Acta Bot. Boreal Occident Sin. **27**:1795-1800.

45Wei, G. P., Zhu, Y. L., Liu, Z. L., Yang, L. F. and Zhang, G. W. 2007. Growth and ionic distribution of grafted eggplant seedling with NaCl stress. Acta Bot. Boreal Occident Sin. **27**:1172-1178.

46Navarro, J. M., Garrido, C., Carvajal, M. and Martinez, V. 2002. Yield and fruit quality of pepper plants under sulphate and chloride salinity. J. Hort. Sci. Biotech. **77**:52-57.

47Calatayud, A., Penella, C., Marsal, J. I., Bonet, L,. Nebauer, S. G. ,San Bautista, A. and Lopéz-Galarza, S. 2013. Empleo del injerto en pimiento como mejora frente a la escasez de agua. Agricola Vergel **366**:212-216.

48Bruce, R. R., Chesness, J. L., Keisling, T. C., Pallas, J. E., Smittle, D. A., Stansell, J. R. and Thomas, A. W. 1980. Irrigation of crops in the southeastern United States: Principles and practices. USDA., Science and Education Administration, Southern Region, Agricultural Reviews and Manuals ARM-S, 9 p.

49Marcelis, L. F. M. and Ho, L. C. 1999. Blossom-end rot in relation to growth rate and calcium content in fruits of sweet pepper (*Capsicum annuum* L.). J. Exp. Bot. **50**:357-363.

50Navarro, J. M., Garrido, C., Flores, P. and Martinez, V. 2010. The effect of salinity on yield and fruit quality of pepper grown in perlite. Sp. J. Ag. Res. **8**:142-150.