



ECOPHYSIOLOGICAL RESPONSES OF POTATO (*Solanum tuberosum*) TO SALINITY AND NITROGEN FERTILIZATION IN SCREENHOUSE, CAMEROON[†]

[RESPUESTAS ECOFISIOLÓGICAS DE LA PAPA (*Solanum tuberosum*) A LA SALINIDAD Y LA FERTILIZACIÓN CON NITRÓGENO EN INVERNADERO, CAMERÚN]

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SUMMARY

Irrigated agriculture under fertilization is a practice that can ensure food security but has the unintended consequence of secondary salinization of arable land. There is the need to forecast production in this increasingly saline environment. An experiment of 4x4 factorial was carried out in screenhouse in Cameroon, to test the responses of potato to salinity under nitrogen fertilization. Plants were grown over two months and standard growth and physiological measurements taken and analysed. Results showed that in conditions of increased sediment salinity up to 12 ppt, tuber yields were greatly suppressed, and did not respond to fertilization. Plants grown under freshwater irrigation had higher root:shoot ratio and tuber development. Plants irrigated with freshwater had higher water use efficiency (WUE), irrespective of whether they were fertilized or not; WUE decreased as salinity increased. Photosynthetic efficiency dropped in all treatments. Under freshwater conditions, potato plants present a more efficient physiology that ultimately results in better growth and tuber development. This results are significant for future potato farming under increasing sediment salinity. In areas where potato is produced, measures should be taken to prevent or reduce the rate of sediment salinization.

Keywords: Secondary salinization; Water use efficiency; Harvest Index; Ecophysiology; Potato

RESUMEN

La agricultura de regadío sometida a fertilización es una práctica que puede garantizar la seguridad alimentaria pero tiene la consecuencia involuntaria de la salinización secundaria en tierras cultivable. Existe la necesidad de pronosticar la producción en este entorno cada vez más salino. Un experimento factorial 4x4 se llevó a cabo en una casa de malla en Camerún, para probar las respuestas de la papa a la salinidad bajo fertilización nitrogenada. Las plantas se cultivaron durante dos meses y se tomaron y analizaron mediciones estándar de crecimiento y fisiológicas. Los resultados mostraron que en condiciones de aumento de la salinidad de los sedimentos de hasta 12 ppt, los rendimientos de tubérculos se suprimieron en gran medida y no respondieron a la fertilización. Las plantas cultivadas bajo riego de agua dulce tuvieron una mayor relación raíz:brote y desarrollo de tubérculos. Las plantas irrigadas con agua dulce tenían mayor eficiencia en el uso del agua (WUE), independientemente de si estaban fertilizadas o no; WUE disminuyó a medida que aumentaba la salinidad. La eficiencia fotosintética disminuyó en todos los tratamientos. Bajo condiciones de agua dulce, las plantas de papa presentan una fisiología más eficiente que finalmente resulta en un mejor crecimiento y desarrollo de tubérculos. Estos resultados son importante para el futuro cultivo de papa en condiciones de salinidad en sedimentos. En las áreas productoras de papa, se recomienda tomar medidas para prevenir o reducir la tasa de salinización de los sedimentos.

Palabras clave: salinización secundaria; Eficiencia en el uso del agua; Índice de cosecha; Ecofisiología; Patata

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a member of the family Solanaceae. Many members of this family including tomato, eggplant, peppers, tobacco, and the

wild night-shade are important food, medicinal, or ornamental plants. In 2010, average world farm yield for potato was 17.4 tons per hectare (FAOSTAT, 2010). In Cameroon, the Northwest and West Regions are the main centres of potato production (Fontem *et*

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al., 2001). Significant production also occurs in the Lebiam Highlands of the South West Region. Potato produced in Cameroon is exported across the CEMAC and ECOWAS markets (Acquah and Lyonga, 1994). The crop has now assumed a cash crop status, with an annual output of 150000 tones, grown on 70000 ha of the national territory. With its status as a cash crop, potato is in high demand within the country, and production barely meets demand. There is also potential for production to meet increasing export needs. However, as with other crops increased production depends on irrigation in the off-season, coupled with the application of fertilizers. This inevitably leads to secondary salinization of arable soils. About 20% of agricultural land worldwide is salinized (Shrivastava and Kumar, 2015), while estimates of salinized crop land are more severe at 50% (Paul and Lade, 2014). Secondary salinization is further compounded by climate change, as increased temperatures increase the rate of precipitation of soluble salts on farmlands (Horie *et al.*, 2012). As arable land becomes more saline, there is need to determine crop plant responses in a view to forecast future production. How potato responds to saline soils is therefore of particular importance. Several authors have studied responses of potato to increasing salinization. Van Hoorn *et al.* (1993) showed that soil salinity resulted in water stress on potato plants, but the overall water use efficiency was not affected. Water use efficiency measures the rate of conversion of irrigation water to plant biomass. Levy (1992) studied salinity effects on different potato cultivars and showed that irrespective of cultivar, growth and tuber yield of potato decreased with increasing salinity. This is the trend generally expected of glycophytes, for example Kumar and Khare (2016) on susceptible and resistant rice cultivars and Blanco *et al.* (2008) on corn. However, nitrogen fertilization is expected to alleviate to some degree, salinity stress in some plants. Hamed *et al.* (2008) showed that growth of *Batis maritima* and *Crithmum maritimum* under saline conditions was limited by restrictions on N uptake at high NaCl concentrations, but there is as yet no clear evidence that augmenting soil nitrogen concentrations would lead to more uptake by plants. At higher salinities, a higher fraction of nitrogen absorbed is diverted to the production of glycinebetaine and/or proline, compatible organic solutes associated with osmotic regulation (Storey *et al.*, 1977, Naidoo and Naidoo, 2001). Few studies of the effects of interaction of these nutrients with salinity on crop plants exist, especially in sub Saharan Africa, with the finality of forecasting future production in current sites, and the need and

possibility of expanding production especially in areas in which potato is not traditionally produced.

The necessity for such studies is now prompted by our varying climates across the world. In the current study the ecophysiological responses of potato to salinity under nitrogen fertilization were studied through its growth and tuber formation, chlorophyll fluorescence as a marker of stress, and water use efficiency, all under the intermediation of nitrogen fertilization.

MATERIALS AND METHODS

The study site

The experiment was conducted in a screen house constructed at SOWEFCU (South West Farmers' Cooperative Union) premises in Kumba, Meme division, which is located at 04.628°58'N latitude and 009.444°98'E longitude, at an altitude of about 237 m asl. Kumba falls within the Cameroon Agro-ecological Zone IV characterized by monomodal rainfall. Mean annual temperature stands at 25 °C and the total average rainfall in the subdivision is about 2200mm (IRAD- Barombi, 2013), and the natural vegetation is equatorial forest. Agriculture is the backbone of the economy, but potato is not among the crops traditionally produced in this agroecological zone.

Green house design and management

The experiment was conducted in a screen house of dimension 16m by 4m. It was constructed with wood and the roof was covered with a transparent polythene to avoid rain. The walls were covered with a mesh, estimated to have adequate light transmission quality and ambient CO₂ levels. Inside of the screenhouse, tables of dimension 4 by 1.5m were constructed with wood at the height of 60cm, to avoid contact with soil. On these tables, experimental pots were placed. During the experiment, temperature within the screenhouse ranged from 24.5 to 41.5 °C with a relative humidity range of 49 to 80.5% at midday.

Source and characteristics of planting material

Seed potato of the Cipira variety (*Solanum tuberosum* L. var Cipira) were obtained from IRAD Dschang, Cameroon. This variety is characterized by oval tubers, brown flesh and is resistant to mildew and viruses and moderately resistant to bacterial swelling. The cropping cycle is 90 to 120 days, with a potential yield of 30 – 35 tons/ha. The seeds were pre-sprouted before planting (Figure 1).



Figure 1: Pre-sprouted seed potato used in the experiment

Experimental design and treatments

There were two factors in this experiment, namely fertilization and salinity. NPK 20:10:10 fertilizer was applied at four levels namely (0, 0.2, 0.4, 0.6 g/plant) representing F0 to F3 respectively. The second factor was salinity, administered at 4 levels namely 0, 4, 8 and 12 ppt. The experimental design was a 4 x 4 factorial experiment with 3 replications, which ran for two months. Salinity treatments were obtained by dilution of seawater of 35 ppt with freshwater.

Planting

Two disease- and pest free tubers of uniform size and single sprouts were planted per 2L pot filled to the brim with homogenized top soil. The soil was clay loam and slightly acidic with a pH of 5.5. The effective cation exchange capacity was 5.45 with base saturation of 15.5%. The percentage organic matter was 3.82% and carbon: nitrogen ratio of 11. The potato tubers were planted so that the buds were directed upward. Where the tuber had more than one shoot, one was retained and the others destroyed. Plants were irrigated with freshwater for two weeks to establish, before treatment application. The plants were subsequently thinned to one plant per pot.

The four rates of fertilizer (0g, 0.2g, 0.4g and 0.6g) were applied two weeks after planting. Irrigation then followed with water of the respective salinity (0, 4, 8 and 12 ppt). All irrigation was done at the rate of 1.2L/pot/week, calculated from the annual precipitation of 2200mm per year.

Weed, pest and disease control

The interior of the screenhouse was cleaned before planting. After sowing weeds were controlled manually on emergence in the pots as well as in the screenhouse entirely. Insects were controlled by the application of Ciperical 12 ec (Cypermethrin 12 g/) three days before planting, throughout the greenhouse. The soil contained in the pots was treated with Ridomil Gold Plus 66 wp (Mefenoxam(Metalaxyl-M) 6%+ CuO 60 %), then by Mancoxyl Plus 720 wp (Cypermethrin 12 g/) one week after planting and two weeks afterwards.

Data collection

Parameters measured over time

Plant height, number of leaves, number of stems, collar diameter, leaf length and width, and chlorophyll fluorescence were measured weekly, between 11 am and 1 pm. Plant height was measured from the base of the plant to the tip of the shoot using a metre rule. Collar diameter was measured using a digital caliper. Leaf and stem numbers were counted, and leaf length and width measured for use in calculating relative leaf area. Chlorophyll fluorescence was measured using a Pocket Plant Efficiency Analyzer (Hansatech Instruments Ltd., Norfolk, UK), after 30 seconds of dark adaptation. The dark adaptation time was determined a priori. Fluorescence parameters measured included the ratio of variable fluorescence and the maximum fluorescence (F_v/F_m) and the performance index (PI). These measurements were performed at midday. During each measuring session, relative humidity and room temperature were measured. For all parameters, the first measurement 2 weeks after planting represented the base measurement.

Parameters measured following harvest

The relative growth rate (RGR) was calculated from the initial and final plant heights as follows (Tabot and Adams, 2012):

$$RGR \left(\frac{cm}{wk} \right) = \frac{LN H_2 - LN H_1}{t_2 - t_1}$$

Where LN = natural logarithm, H2 = final height, H1 = initial height, t = time in weeks.

At harvest, number of tubers, tuber yield, root:shoot ratio, harvest index and water use efficiency were determined. Number of tubers were counted per

treatment and tuber yield determined by weighing on a sensitive balance. The ratio of the roots to the shoot was calculated after separation of roots from shoots, and carefully cleaning both (Tabot and Adams, 2012):

$$\text{Root: shoot ratio} = \frac{\text{root mass (g)} + \text{tuber mass (g)}}{\text{shoot mass (g)}}$$

The harvest index was calculated as follows (Amanullah and Inamullah, 2016):

$$\text{Harvest Index} = \frac{\text{Tuber mass (g)}}{\text{Total Plant mass (g)}}$$

Water use efficiency (WUE) was calculated as the ratio between the total plants biomass (g) produced and the total amount of water (L) applied during irrigation (After Egbe *et al.*, 2014).

$$\text{WUE} \left(\frac{\text{g}}{\text{L}} \right) = \frac{\text{total plants biomass}}{\text{Total amount of water applied}}$$

Data analysis

Data were analyzed for variance using the GLM ANOVA function, after tests for normality and homogeneity of variance. Means were separated through Tukey HSD test. Because some parameters were not normally distributed, Spearman Rank Correlation analyses were done to examine relationships between parameters. All analyses were done at $\alpha = 0.05$ using Minitab® version 17 statistical package (Minitab Inc, CA, USA).

RESULTS

Table 1 shows the significance of main and interaction effects of parameters measured over time. For the growth parameters, there were statistical differences in height in response to salinity, fertilization and time. The effect of interaction of salinity and fertilization on height was also significant ($p < 0.05$). The response of

collar diameter to treatments and interaction effects was similar to that of height (Table 1). Number of stems varied significantly under treatment with salinity, and increased with time, and the interaction of salinity and fertilizer on stem number was also significant. For the main effects, salinity significantly affected leaf area, while the effect of fertilization was not significant. Chlorophyll fluorescence (fv/fm) was not significantly affected by treatments, but varied significantly over time.

Table 2 is of the significance of responses of root:shoot ratio, water use efficiency, number of tubers, tuber yield, harvest index and relative growth rate to salinity, fertilization and the resulting interaction. At $\alpha = 0.05$, salinity significantly influenced root: shoot ratio and water use efficiency; tuber numbers, tuber mass and harvest index were significantly affected at $\alpha = 0.001$. Harvest index varied significantly with fertilization, and the interaction between salinity and fertilization significantly affected all yield parameters (Table 2). Relative growth rate of potato plants was significantly affected by salinity treatments.

Responses of parameters to salinity

Figure 2 details responses of variables to salinity. Plant height was statistically similar at 0 and 4 ppt but plants treated with 8ppt saline water were significantly taller. This is reflected in the RGR (Figure 2 B). Leaf area decreased significantly as salinity increased from 0 to 4 ppt, with a slight increase at higher salinities. Chlorophyll fluorescence decreased quantitatively as salinity increased from 4ppt to 12 ppt but this difference was not statistically significant. Root:shoot ratio was highest (1.76) in plants irrigated with freshwater, and decreased significantly as salinity increased to 12ppt. Tuber yield measured as fresh tuber mass decreased sharply with an increase in salinity. The heaviest tubers (20.36g) were produced by plants irrigated with freshwater Figure 3. Tuber yield decreased thereafter, to 0.38g in plants irrigated with water of 12 ppt salinity (Figures 2 and 3).

Table 1: Significance of main and interaction effects of salinity and fertilization on height, number of stems, leaf area and chlorophyll fluorescence of potato measured over time in screenhouse, Cameroon

Source	Height	No. of Stems	CD	Leaf area	fv/fm
Salinity (S)	***	***	***	***	ns
Fertilization (F)	*	ns	***	ns	ns
Time (T)	***	***	***	***	***
S x F	**	***	***	ns	ns
S x T	ns	ns	ns	ns	ns
F x T	ns	ns	ns	ns	ns
S x F x T	ns	ns	ns	ns	ns

CD = collar diameter, fv/fm = ratio of variable to maximum chlorophyll fluorescence, * = significant at $\alpha = 0.05$, ** = significant at $\alpha = 0.01$, *** = significant at $\alpha = 0.005$, ns = not significantly different

Table 2: Significance of main and interaction effects of salinity and fertilization on height, number of stems, leaf area and chlorophyll fluorescence of potato measured over time in screenhouse, Cameroon

Source	Root:Shoot ratio	WUE	No. tubers	Tuber yield(g)	HI	RGR
Salinity (S)	*	*	***	***	***	*
Fertilization (F)	ns	ns	ns	ns	*	ns
S x F	ns	ns	**	**	***	ns

WUE = water use efficiency, HI = Harvest Index, RGR = relative growth rate, * = significant at $\alpha = 0.05$, ** = significant at $\alpha = 0.01$, *** = significant at $\alpha = 0.005$, ns = not significantly different

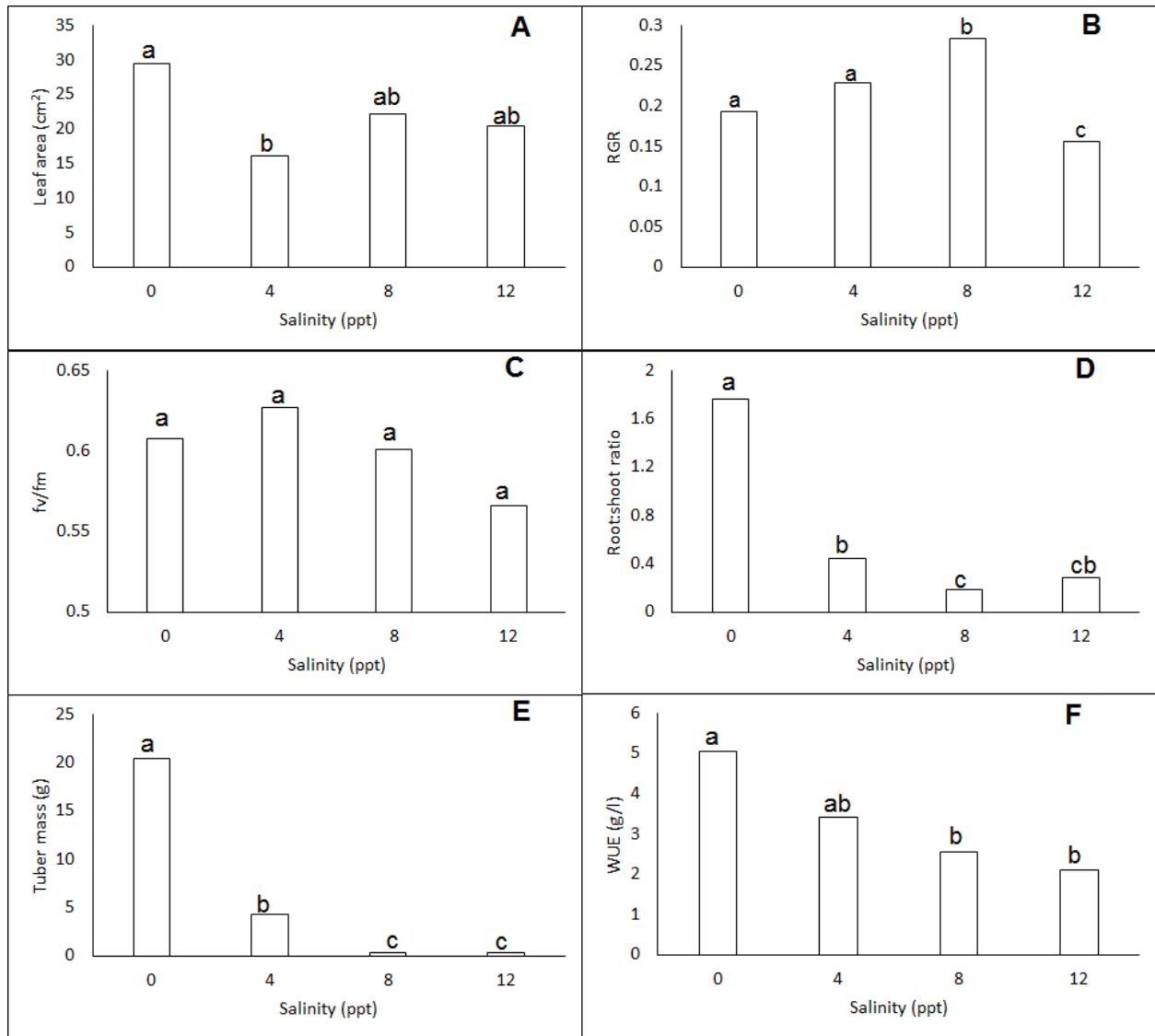


Figure 2. Responses of measured variables to salinity treatments. A =leaf area, B= plant height, C= chlorophyll fluorescence, D=root/shoot ratio, E=tuber mass and F=water use efficiency. Bars represent means. Means separated through ANOVA with Tukey pairs wise comparisons at $\alpha=0.05$. Bars with the same letter for each variable are not significantly different.



Figure 3 Tubers per plant harvested after 8 weeks of salinity treatment in greenhouse. Salinity treatments ranged from 0 ppt to 12 ppt

Responses of parameters to fertilization

Plant height increased significantly with increase in fertilization, from 35.7cm in plants that were not fertilized, to 48cm in plants treated with 0.6g fertilizer. There was no significant fertilizer effect on leaf area, chlorophyll fluorescence, root:shoot ratio and water use efficiency (data not presented).

Effects of interactions between fertilizer and salinity on potato responses

The effects of the interaction between salinity and fertilization on parameters measured are presented in Figure 4. As seen from Tables 1 and 2, these interactions had a significant effect on plant height, tuber yield and Harvest index. At 0ppt, plants that were fertilized were taller than those not fertilized. At higher salinities, height of unfertilized plants was statistically comparable to those of fertilized plants. At all fertilizer levels, there was higher tuber yield from pots irrigated with freshwater and yield decreased sharply irrespective of fertilization as salinity increased. At 4ppt, the unfertilized plants had better yield than the fertilized plants. With respect to water use efficiency, plants irrigated with freshwater and supplied 0.2g fertilizer had the best WUE (8.61g/l) while the least was in unfertilized plants irrigated with water of 12 ppt

salinity (1.3g/l). Fertilized plants at this high salinity had better WUEs (Figure 4).

Figure 5 shows the interaction of plant height with chlorophyll fluorescence over time. Over time, as plant height increased, f_v/f_m decreased. Beyond the 5th week, plant height also began to experience a decrease. There was a mild negative correlation between plant height and f_v/f_m ($\rho = -0.197$, $p = 0.001$) but no correlation with RGR.

Correlations

There were significant negative correlations between WUE, number of tubers, tuber mass, biological yield and harvest index with salinity. Some of these correlations are presented in Figure 6. There were no significant correlations with fertilizer. Root shoot ratio and WUE correlated positively with related yield parameters. Growth parameters measured over time (data not shown) seem to be negatively affected by salinity, but indeed the only negative correlations were between salinity and number of stems ($\rho = -0.197$, $p = 0.001$). Plant height, number of stems and number of flowers were significantly positively related to time ($\rho = 0.488$, 0.225 , 0.145 respectively, $p < 0.05$), while chlorophyll fluorescence correlated negatively with time ($\rho = -0.536$, $p = 0.000$).

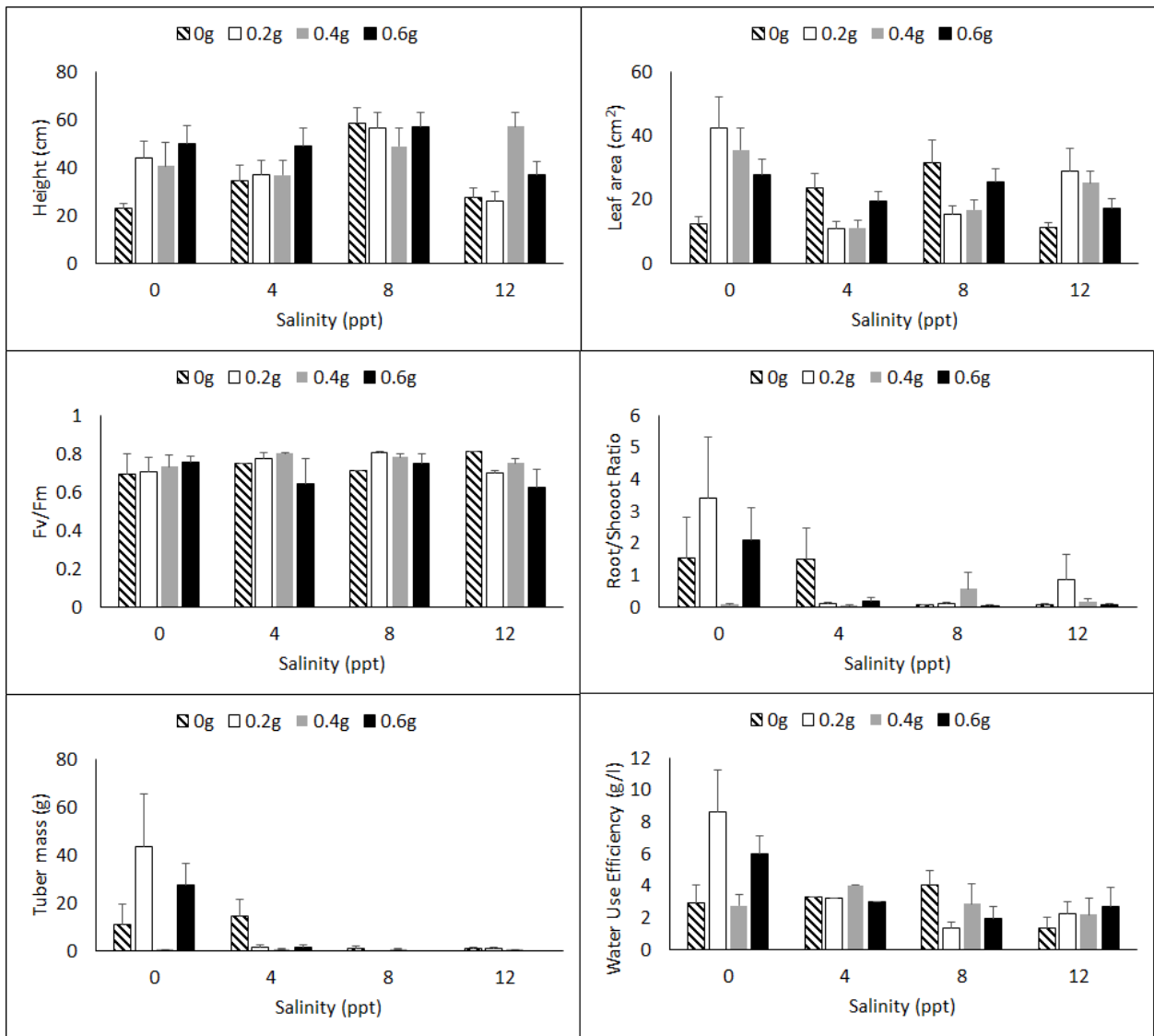


Figure 4. Responses of plant height, leaf area, chlorophyll fluorescence, root:shoot ratio, tuber yield and water use efficiency of potato to fertilization under four salinity regimes in screenhouse, Cameroon

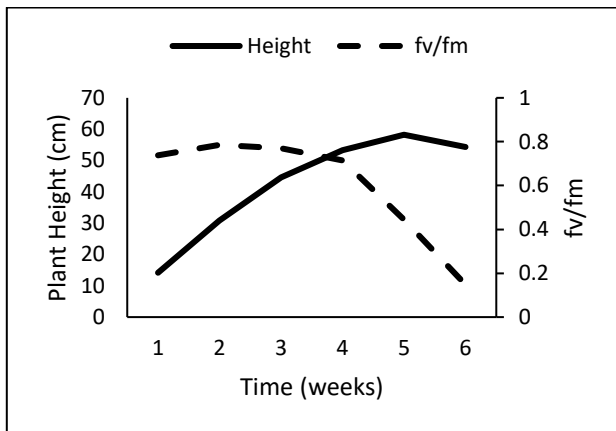


Figure 5. Relationship between fv/fm and plant height over 6 weeks in screenhouse, Cameroon

DISCUSSION

Morphological responses to salinity and fertilization

Statistically, salinity effects on growth parameters were significant, but the pattern is not clear; plant height were stimulated up to 8ppt, while leaf area decreased as salinity increased from 0ppt. For glycophytes, the expected result is a decrease in growth with salinity increase (Horie *et al.*, 2012). Glycophytes like potato lack mechanisms of tolerance to salinity like ion compartmentalization, salt secretion etc. However, the very similar heights and leaf area show that for the first six weeks of growth, potato grown in screen house was more tolerant of salinity than expected. This could be due to counteracting effects of nitrogen fertilization. It has been shown that

nitrogen fertilization augments plant growth and yield (Muhammad *et al.*, 2015; Saravia *et al.*, 2016). We intended to find a role for nitrogen in mediating salinity stress. One way in which salinity stress affects plants is inhibition of nutrient uptake (Munns and Tester, 2008). Incidentally, nitrogen is a key component of most systems that tolerate salt stress. The fact that nitrogen addition did not translate to increased growth suggests that it went into systems that improve potato tolerance to salinity. Nitrogen is a key component of proline and glycinebetaine, essential compatible solutes that are responsible for salinity tolerance, as they scavenge reactive oxygen species and stabilize cell membranes (Naidoo and Naidoo, 2001; Munns and Tester, 2008; Tabot and Adams, 2012). The levels of these solutes were not assessed in this study, but other authors have already established their role in other plants (Naidoo and Naidoo, 2001; Tabot and Adams, 2012). The contribution of Nitrogen is evident in the WUE, where fertilized plants at 12ppt had better WUE than unfertilized ones. While nitrogen

fertilization might have improved plants' tolerance to salinity, tuber yield in number and mass decreased significantly as salinity increased from 0ppt; there were significant negative correlations between salinity and tuber yield, tuber number, harvest index and other parameters dependent on tuber yield, but no correlation of these parameters with fertilization. This suggests the salinity stress is predominant and only mildly moderated by Nitrogen fertilization. Salinity typically increases oxidative stress in plants including water stress and restrictions in nutrient uptake (Munns and Tester, 2008). The result is that photosynthesis efficiency is reduced (Redondo-Gómez *et al.*, 2007), and much of the photosynthate is diverted to stress tolerance mechanisms, with little left for the sinks. Tuber formation is thus suppressed, as is the harvest index. Therefore, in conditions of increased sediment salinity, it is expected that potato growth could persist if supplemented with nitrogen fertilization, but tuber yields will be greatly suppressed.

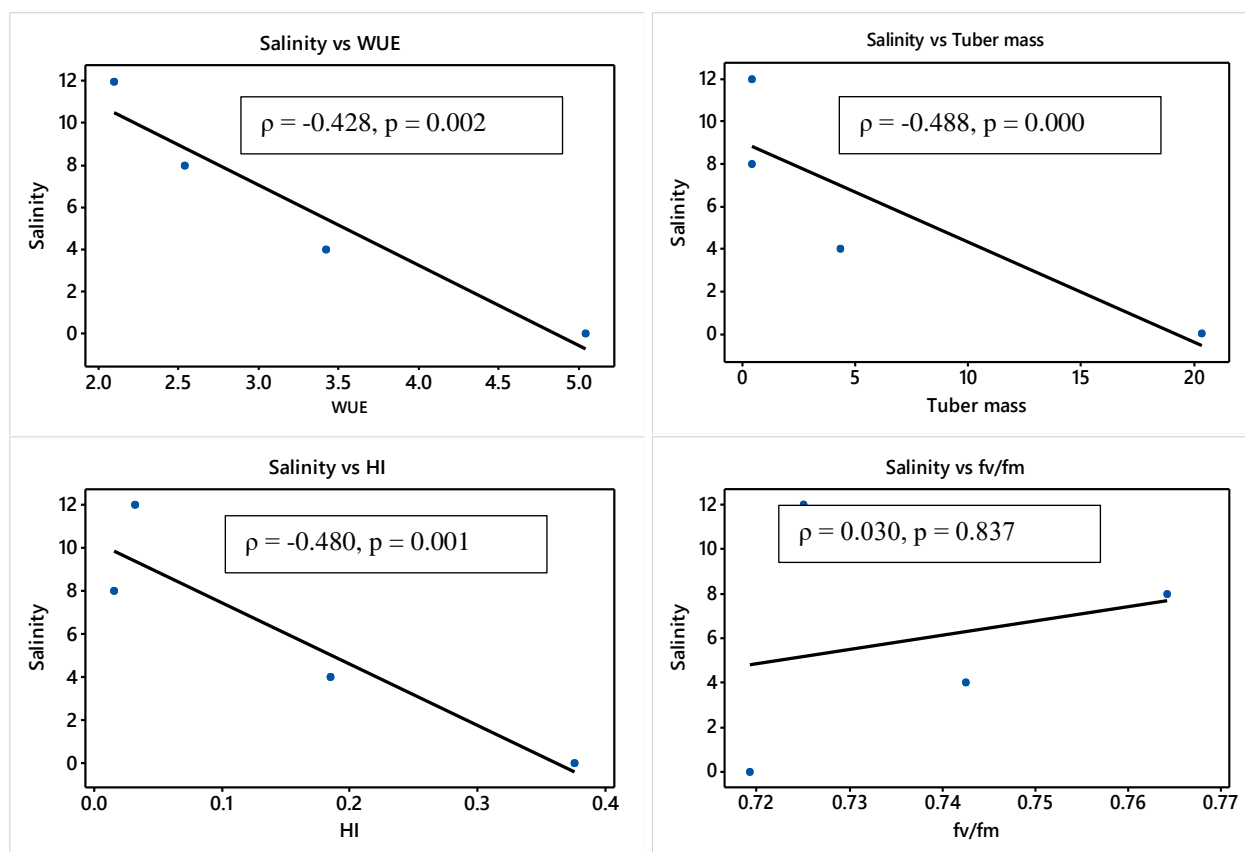


Figure 6 Correlations between salinity and water use efficiency, tuber mass, harvest index and chlorophyll fluorescence. ρ = Rho, the Spearman Rank Correlation coefficient; p = the level of significance.

Physiological responses to salinity and fertilization

Chlorophyll fluorescence measured as an index of the efficiency of photosynthesis did not vary significantly with any main effect but decreased significantly over time. The ratio f_v/f_m was consistently below 0.8, which is the expected threshold for healthy unstressed plants, but above 0.3, which is the value of photosynthetic efficiency beyond which plants are deemed non-viable and would most possibly die (Woo *et al.*, 2008). This shows that under salinity treatments, potato plants were stressed across the board and this stress is bound to result in reduced productivity. Similar stress levels across salinity treatments suggest that other environmental conditions affected plants in all treatments. The RH was often low and greenhouse temperatures high, which themselves contribute to stressing the plants. Salinity-stressed plants typically exhibit higher root:shoot ratios, as photosynthate is diverted to improve root architecture for enhanced nutrient uptake (Tabot *et al.*, 2012). However, in the present study, potato plants grown under freshwater irrigation had higher root:shoot ratio, as the conditions were more favourable for root and tuber development; if the plant were tolerant of salinity we would expect similar root:shoot ratios at the higher salinities but this decreased sharply. Similarly, WUE varied significantly with salinity but not fertilization. Plants irrigated with freshwater had higher water use efficiency, irrespective of whether they were fertilized or not. Water use efficiency gives an indication of the plants' ability to convert irrigation water into biomass (Egbe *et al.*, 2014). Since the potato was the same variety we expected similar WUE, but this decreased as salinity increased. Under freshwater conditions, potato plants present a more efficient physiology - more efficient photosynthesis, better water balance, better gaseous exchange and more efficient moisture and nutrient uptake that ultimately results in better growth and tuber development, and more efficient use of water.

CONCLUSION

Our results are significant for the future of potato farming in an environment of increasing salinity of arable land. While salinity might stimulate growth in height of plants, it drastically reduces tuber formation, and supplementation with nitrogen fertilization is not enough to counter the retarding effects of sediment salinization. Therefore, in potato producing areas, measures should be taken to prevent or reduce the rate of sediment salinization.

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