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Effect of Nitrogen on Agronomic Yield, Spad Units and Nitrate Content in Roselle (*Hibiscus Sabdariffa*l.) in Dry Weather

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Abstract— *This study was conducted in polyethylene bags of 4 kg capacity with plants of Jamaica from seeds collected from an accession on the coast of Oaxaca, which were planted under the ecological conditions of Teotitlán de Flores Magón, and evaluated under completely randomized design, where treatments were four levels of nitrogen: 0, 50, 100 and 150 kg ha*$^{-1}$ *and four repetitions (4x4) = 16 experimental units. The variables evaluated were: both agronomic yields: chalice and seed, harvest index, SPAD units and nitrate content in leaf. The results indicate that higher yields ofseed and chalice, biomass, nitrate content in leaf and SPAD units were achieved with the application of 100 kg ha*$^{-1}$ *of nitrogen with 50.39, 196.80, 620.4 g plant*$^{-1}$*, 85.00 mg kg*$^{-1}$ *and 29.10 units, respectively. The content of nitrates and its relationship with SPAD units, adjusted to an increasing linear model for the four levels of nitrogen studied. From this study it can be concluded that the application of 100 kg N ha*$^{-1}$*, positively affect the culture of Jamaica under dry weather conditions way.*

Keywords—harvest index, chalice, biomass, accession.

I. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is a plant belonging to the family of the malvaceae (Gonzalez, 2014; De Dios *et al.*, 2011),its center of origin is in the center Abyssinian (Ethiopia and Somalia),although some authors as Vavilov (1920), It mentions that its center of origin is Sudan on the African continent (León, 2003).This plant for many years has been cultivated in tropical areas of the world,to make a refreshing drink,although it has also been used in traditional medicine as a diuretic being considered,even been shown to have antimicrobial and antioxidant properties (Pabón*et al.*, 2013),further pigments which are intended for food are extracted,also it used in the production of jams (Cid and Guerrero, 2012).Pascualides*et al.*, (2013) mention that the genus *Hibiscus* has other uses such as:paper pulp, biofuel, forage and extraction of oil from its seeds.Nitrogen, is regarded as an essential element in the growth and development of crops, because it is part of macromolecules such as amino acids, proteins, chlorophyll (Cardenas *et al.*, 2004). When it is missing,chlorosis appears on the leaves of the plant,besides producing small leaves and little development in general on the plant (Marquez *et al.*, 2013),it has been shown that nitrogen,reduces crop yield due to the reduction in leaf size,why photosynthesis is limited as well as the yield (Vazquez *et al*., 2008).Under this trend,It has had to implement techniques,that provide insight into the nutritional status of crops when they are fertilized mainly with nitrogen (Rangel-Lucio *et al.*, 2002).So, it has been shown that nitrogen positively affects yield components,number of pods, seeds, protein concentration and biomass allocation in some crops such as beans and faba-bean (Escalante*et al.*, 2015;Guadarrama*et al.*, 2007).One strategy is to know the SPAD units,measuring the intensity of green color of the leaves of the crops,shown a positive correlation between the content of N,as nitrates and SPAD units,as demonstrated in maize,where yield is related to the SPAD units (Gonzalez *et al.*, 2009). Another use of the SPAD units, It is closely related to chlorophyll content, so Varvel *et al.*, (2007),mentions that the Minolta SPAD-502 meter units,It can be used as an indirect measure of the chlorophyll content in the crops.

For this reason,in this study the objective was:determine the yield and seed roselle, chalice according to four levels of nitrogen,and their relationship with SPAD units and nitrate content in leaf, under dry weather.

The hypothesis was:different nitrogen levels affect agronomic yield, biomass, SPAD units, and nitrate content in leaf cultivation of roselle.

II. MATERIALS AND METHODS

- **Location of the experiment.**

This study was conductedduring the period June-September of 2015 inTeotitlan Flores Magon, Oaxaca Mexico. Town located 18º 08' 00" north latitude, 97º 05' 00" west longitude and 888 meters of altitude, under a climate Bs_1(w ')(w)egwhich corresponds to a dry climate with more than 18°C and 27°C lower than annual average temperature,with total precipitation greater than 400 and less than 600 mm,The rainy season runs from June to September,intraestival presence of drought,the oscillation of temperature is greater than 7°C and less than 14°C between the warmest and the coldest month,the warmest month is presented before the summer solstice,in the area it occurs in April (Garcia, 2005).To have better control of the experiment,one ombrothermic diagram to see maximum temperatures fluctuate is did,and minimal rainfall during the crop cycle.

- **Germplasm.**

The genotype used,seeds were collected from an accession open-pollinated on the big coast of Oaxaca, It is corresponding to chalice red and white corolla gamopetalous.Which were planted on June 9 of 2015,in polyethylene bags four kg capacity depositing two seeds per bag.

- **Physical and chemical characteristics of the soil.**

Soil area was used,corresponding to an epileptic luvisol in formation with colluvial remains,whose physicochemical characteristics are presented in Table 1.Nitrogen values from the previous analysis,They were used to adjust the different fertilization.The experimental units were placed directly on the ground in the open.

Table.1.Physical and chemical properties of an epileptic luvisol, in Teotitlán de Flores Magón Oaxaca, Mexico.Sown with roselle (Hibiscus sabdariffa L.)Summer 2015.

Physicalproperties		**Chemicalproperties**	
Apparentdensity	1.26 g cm^{-3}	Nitrogen	7.1 mg kg^{-1}Kjendahl¶
Texture	Franco-Shale	Phosphorus	4.3 mg kg^{-1}Olsen
		pH	7.4
		E. C.	2.9 dS m^{-1}
		O. M.	1.8 %
		Saturated Bases cMol$^{(+)}$ kg^{-1}	
Color		Na^+	0.2
Dry	10R 6/8	K^+	0.8
Damp	10R 4/6	Ca^{++}	3.2
		Mg^{++}	0.8

E. C., electric conductivity; O. M., organic matter.
¶

The above parameters were described using the following methods:

- pH, in water by the potentiometric method with the ratio soil: water 1:2.
- Organic matter by Walkley& Black method.
- Interchangeable bases by the extraction method with ammonium acetate at pH 7.0
- Mechanical composition by the method of Bouyoucos, using sodium hexametaphosphate as a dispersant.
- Phosphorus assimilable by the Olsen method for soils with pH greater than 7, available potassium by calculation from the exchangeable potassium (NOM-021-RECNAT-2000).

- **Treatments and experimental design.**

Treatments were four:0, 50, 100 and 150 kg $ha^{-1}$of Nitrogen,whose source was urea (46% N),which is applied at 30 days after sowing the seedlings had when a height of 20 cm.These were evaluated under a completely randomized design (DCA) and four repetitions (4x4) = 16 experimental units,analyzed under the mathematical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ where:Y_{ij}, is the response variable i-th level of nitrogen in the j-th repetition; μ, it is the true overall average;T_i, it is the effect of the i-th level of nitrogen andε_{ij}, is the error of the i-th level of nitrogen in the j-th repetition. The experimental unit, It consisted of the polyethylene bag more soil over the plant (Cochran and Cox, 1990).

- **Variable response.**

Agronomic yield,was determined for seed, chalice and weighing the total seed and chalice separately per plant,with an analytical balance OBI-207134 and expressing the result in grams per plant (g plant^{-1}).Total biomass per plant,subjecting drying structures: stems, leaves, seed and caliculos, in a forced air stove for 72 hours,up to the constant weight and weighing these structures in an analytical balance to express the results in (g plant^{-1}).Harvest index, using equation HI $= \frac{AY}{BY}$, where: HI, is the harvest index; AY, agronomic yield, which may be the weight of chalice or seed, BY is the total plant or biological yield (Morales et al, 2008) dry weight.Nitrate content in leaves,for this variable a sample of 5 g of fresh leaves was taken,which were finely minced were blended in 100 ml of deionized water,to further filter and make reading the content of nitrates in the UV-visible spectrophotometer tape 10e,at a wavelength of 520 nm (Escalona*et al*., 2009). SPAD units, these were measured directly on the sheet using a meter Minolta SPAD-502 units,readings were made at noon,V_7 in the vegetative stage corresponding to the plant when it has 8 true leaves and is pre-anthesis, the average height of the population at this stage was 90 cm.For the relationship between nitrogen,agronomic yield and SPAD units,Mathematical models were developed to determine the degree of association between these variables,and the respective coefficient of determination.The response variables were analyzed with the statistical package SAS 9.1.3 Portable, when they are significant ($P\leq 0.05$),the multiple comparison test of Tukey at a significance level of 5% probability of error applies.

III. RESULTS AND DISCUSSION

- **Ombrothermic diagram.**

The maximum temperature, minimum and precipitation that make up the diagram ombrothermic during ontogenetic crop cycle,are presented in Figure 1,it can be seen that the maximum temperature was between 47 and 37^{o}C,while the minimum between 14 and 20^{o}C.Regarding the monthly sum precipitation was 292.00 mm,the largest percentage of this occurred during the month of September with 94.0 mm corresponding to 32.19%, likewise in the graph shows very marked intraestival drought,which occurred during the period from July 20 to August 25.It's important to mention,under these conditions the crop reached physiological maturity smoothly,thus having a cycle of four months covered from June to September.

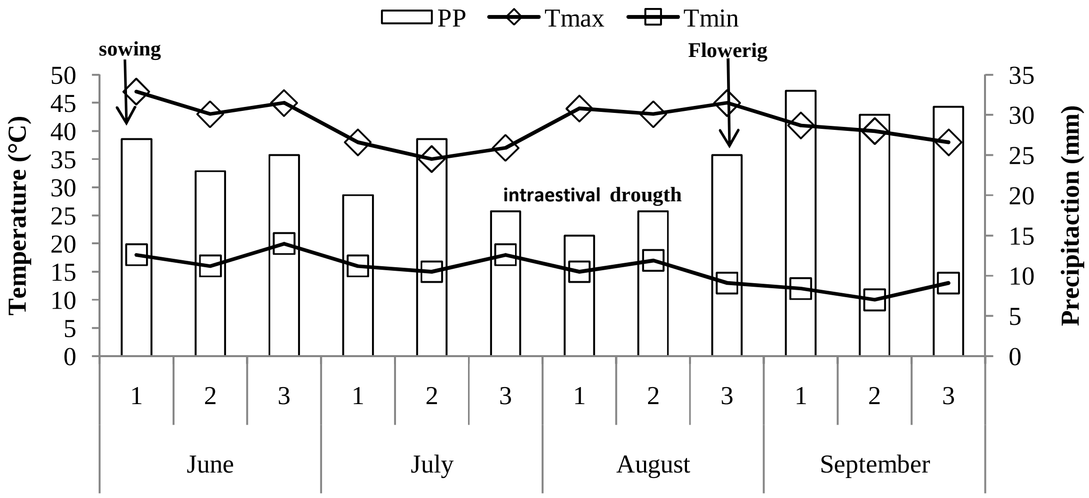

Figure.1: Maximum temperature, minimum and precipitation during ontogenetic cycle roselle (Hibiscus sabdariffa L.) based on four levels of nitrogen,in Teotitlán deFlores Magón Oaxaca, México. Summer 2015.PP, precipitation; Tmax and Tmin, temperatura maximum and minimum respectively.PP, precipitation; Tmax, maximum temperature; Tmin, minimum temperature.

- **Agronomic yield and harvest index.**

The chalice and seed yields and harvest rates differ significantly by the effect of the dose of nitrogen. The coefficient of variation for all variables ranged between 19.00 and 26.83 %, indicating that the data were reliable,to not have a high variability.Regarding the means test, the highest yield of chalice was to high doses of T_{150} and T_{100} N with 205.33 and 196.80 g plant^{-1} respectively, while the lowest yield was chalice to the witness with 16.33 g plant^{-1} (Table 2).Seed yield, has a trend similar to chalice,as higher yields occurred at high doses with 60.44 and 50.39 g plant^{-1}.The harvest index was a reflection of what happened in the chalice and seed yield,as the highest values were recorded with the application of 150 and 100 kg N ha^{-1}, with 0.32 and 0.31 for chalice and seed values of 0.09 and 0.08 were recorded respectively. This indicates that with the addition of 150 and 100 kg ha^{-1} of N in growing roselle,32 and 31% of the biomass produced in the process of photosynthesis, is assigned directly in the chalice,while with these doses of nitrogen in the seed is assigned only between 9 and 8% from carbohydrates.This study differs from that reported by Golik*et al.*, (2003), who report a HI of 0.73 in wheat under conditions of La Plata, Argentina,value that contrasts sharply with the data presented here despite being of small seed species like nitrogen fertilization under 100 kg ha^{-1}.This phenomenon may perhaps be due in large part to the different studies where the studies were conducted, as well as different climate zones and especially the difference between species.For its part Morales *et al.*, (2007),reported a rate of 0.31 in sunflower crop when it is sown in monoculture and the application of 100 kg N ha^{-1},fact consistent with the present study, despite being from different species.

Table .2: Analysis of variance and multiple comparison test four variables in response to four levels of nitrogen, in Teotitlan de Flores Magon Oaxaca, Mexico. 2015.

Treatment	**Yield (g plant^{-1})**		**H.I**	
	Chalice	Seed	Chalice	Seed
T_0	65.80 c¶	16.33 b	0.12 b	0.03 b
T_{50}	110.70 b	28.45 b	0.20 b	0.05 ab
T_{100}	196.80 a	50.39 a	0.31 a	0.08 a
T_{150}	205.33 a	60.44 a	0.32 a	0.09 a
HSD	22.10	19.50	0.08	0.03
Varianceanalysis	*	*	*	*
CV %	26.83	20.30	19.00	22.15

¶Means within columns with the same literal are statistically equal according to Tukey P≤0.05;T_0, T_{50}; T_{100}; T_{150}, treatments; H. I., Harvest index; C. V., Coeficiente of variance; **;*; n.s., significant at 0.01; 0.05 and no significant.

- **Total biomass.**

Biomass significant differences between treatments,the largest allocation is for T_{100} and T150 with 620.40 and 630.22 g plant^{-1} respectively, without statistical differences,surpassing the T_{50} and control treatments,which despite having numerical differences are statistically equal with 550.26 and 526.30 g plant^{-1}.This behavior corresponds to that in the treatments where there was a greater contribution of N,the plant responded by allocating a greater amount of biomass,this response was due to the treatments where more nitrogen was applied, plants tend to display greater leaf area,thus promoting greater intercepted radiation and therefore a greater allocation of photosynthates as biomass (Diaz *et al.*, 2007; Meléndez*et al.*, 2006).

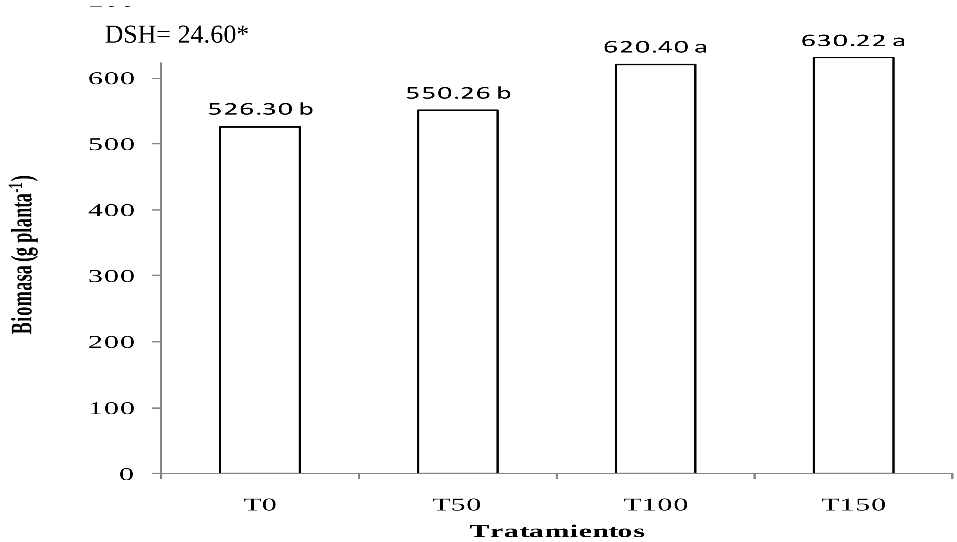

Fig.2: Comparison Test averages for total biomass in roselle (Hibiscus sabdariffa L.) under four levels of nitrogen, in Teotitlán de Flores Magon Oaxaca, México. 2015.

- **Nitrate content and SPAD units.**

The nitrate content of fresh leaf extract is presented in Table 3,the highest content 91.00 and 85.00 mg kg^{-1} corresponds to the T_{150} and T_{100} treatments, resulting to be statistically equal and beating treatments T_{50} and $_{T0}$ who only contain 77.00 and 30.00 mg kg^{-1} respectively.The study presented in this, is similar to the results obtained by Amador *et al*., (2005),who mentioned differences in the concentration of cellular extract of petiole,with increasing concentration of N 0, 50 and 100 mg L^{-1} in the solution Steiner, in four hybrids of jalapeño pepper, but they differ in nitrate concentration of petiole extract,as it reported a maximum concentration of 1050 mg L^{-1} NO_3^-.These differences are due,the different species in which both studies and concentrations of N used are developed.

Table.3: Analysis of variance and comparison of averages for nitrate content and SPAD units in response to four levels of nitrogen in roselle (Hibiscus sabdariffa L.), in Teotitlan de Flores Magon Oaxaca, Mexico. 2015.

Treatment	NC $_{(fresh\ matter)}$ mg kg^{-1}	SPADunits
T_0	30.00 c	20.71 b¶
T_{50}	77.00 b	27.80 b
T_{100}	85.00 a	29.10 a
T_{150}	91.00 a	30.12 a
DSH	9.30	4.50
Varianceanalysis	*	*
CV %	16.70	16.44

¶Medias within columns with the same literal statistically equal according to Tukey P≤0.05; T_0, T_{50}; T_{100}; T_{150}, treatments; NC, content of nitrates; CV, coefficient of variation; *; **; n.s, significant at 0.05; 0.01 and not significant.

- **Relationship between nitrate and SPAD units.**

The nitrate content in the leaf,a linear model was adjusted in a range of 20 to 30 SPAD units. So the slope of the curve was, 6.5 indicating that SPAD per unit increased,the nitrate content in the leaf increases at 6.5 mg kg^{-1}(Figure 3).This has been corroborated by Loewy and Ron (2009), who mentioned that the SPAD units with each unit increase wheat nitrogen supplied to crops.Similarly indicate that these units,in a range of 25 to 30 indicates a very low level of N,this contrasts with the results obtained in this investigation because these authors say the SPAD values 25 to 30 are low in wheat cultivation,fact that did not influence Jamaica cultivation as this reached physiological maturity without problems.This phenomenon can be attributed to the difference in pigment,having leaves roselle,as anthocyanins and carotenoids in photosynthesis performing different wavelengths which does chlorophyll (Taiz and Zeiger, 2002).

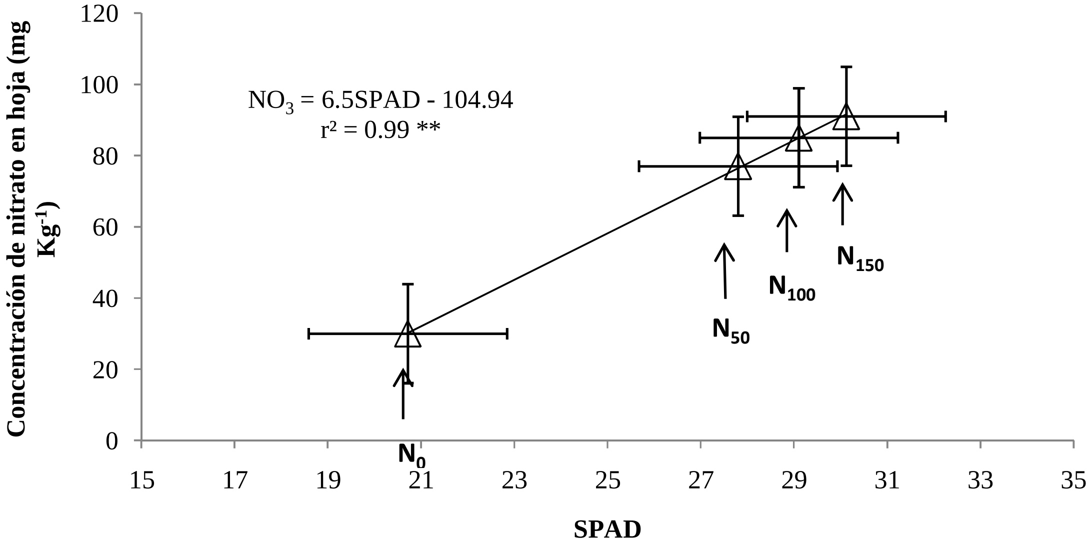

Fig.3: Relationship between SPAD units and nitrate concentration in leaf of roselle (Hibiscus sabdariffa L.), according to four levels of nitrogen in Teotitlán de Flores Magon Oaxaca, Mexico. 2015.

- **Nitrate ratio seed yield and chalice.**

The yield tuning chalice and seed,SPAD regarding units in both cases, He was linear presenting a high coefficient of determination 0.86 for seed, while the yield of the chalice,presented a determination coefficient of 0.80, Thus, the slope for seed yield was 4.1, indicating that for every SPAD unit that increases seed yield increases 4.1 g plant^{-1}, while the slope for chaliceyield was 14.1, showing that for every SPAD unit increases are obtained 14.1 g plant^{-1}. These results can be used to predict both seed yield and chalice,starting reading units using the SPAD-502 Minolta.The results that came in this study,They are consistent with those reported in small grains (wheat) by Gandrup*et al.*, (2004), those working with the Minolta-502 to predict yields in this crop,Using the greenness index and the rate of nitrogen deficiency obtained determination coefficient values of 0.70 and 0.84,and mentions that the settings are a good estimator to predict grain yields in wheat cultivation.

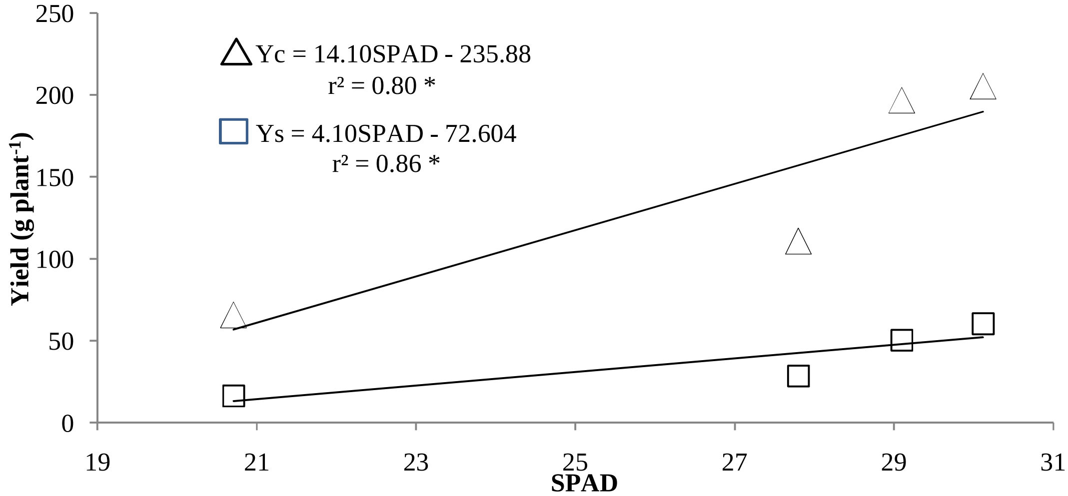

Fig.4: Seed yield and chalice in roselle (Hibiscus sabdariffa L.) based on four levels of nitrogen, in Teotitlan de Flores Magon Oaxaca, México. 2015. Yc, Yield of chalice; Ys, yield of seed.

IV. CONCLUSIONS

Open-pollinated germplasm used in this investigation, it responded differently to the levels of nitrogen supplied way,so the highest seed yield, chalice, biomass and nitrate content were high applications with 100 and 150 kg N ha^{-1}.The SPAD units,proved to be useful in determining the content of nitrates in leaf,and similarly they can be an estimator for determining chalice and seed yield.

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