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# CRITICAL NITROGEN CURVE FOR TWO POTATO CULTIVARS UNDER SEMI-ARID CONDITIONS

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#### Abstract

Plant based analytical techniques of nitrogen nutrition, established on the concept of critical nitrogen can be used to diagnose the nitrogen (N) status of potato, which in turns can provide understanding of N nutrition and serve as a guide for the profitability and sustainability of agricultural production system in semi-arid conditions. Critical N concentration (Nc) was determined from three on-farm field trials in which two cultivars (Spunta and Bellini) were grown under three or four N rates. Dry matter and N concentration of shoots and tubers were measured. Nc was determined by selecting the minimum N concentration for which the highest total biomass (W), comprised of shoots and tubers, was obtained and by expressing N concentration as function of W. Nitrogen nutrition index (NNI) was determined as the ratio between measured N concentration and predicted Nc, was then related to the relative yield (RY) measured at harvest. The allometric relationship between N concentration and W for Spunta (Nc =  $3.25W^{-0.31}$ , R<sup>2</sup> = 0.99) was different from that of Bellini (Nc =  $2.99W^{-0.38}$ , R<sup>2</sup> = 0.93) indicating that Spunta had a greater N concentration than Bellini. These results suggest that there is intra-specific variability in potato for the relationship between N concentration and biomass. Relationships between RY and NNI of Spunta and Bellini were expressed by linear functions and accounted for 51% and 66% of variation, respectively. Our results suggest that NNI could be a reliable indicator of the level of N stress during the growing season of Spunta and Bellini.

**Keywords:** *Potato, Critical nitrogen concentration, Nitrogen nutrition index, Relative tuber yield, Semi-arid conditions.* 

### Introduction

Plant based diagnostic methods of N deficit can be used to improve the efficiency of N utilization and diminish the risks of N losses to the environment. These diagnostic methods should be based on the concept of N concentration that is the minimum N concentration required to achieve maximum crop growth (Ulrich, 1952). It is well established that the N concentration in many crops decreased with increasing plant biomass (Greenwood et al., 1990). The progressive decline of %N in shoot and tuber biomass is attributable to plant compartmentalization. Plant N content varies according to a metabolic pool, associated with photosynthesis and growth process, and a structural pool corresponding to N storage, structure and other metabolic functions. N dilution is due to two processes: self-shading of leaves and change in the leaf:shoot ratio during crop development (Yao et al., 2014). This decline in N is described by a negative power function called dilution curve. The critical N (Nc) dilution curve can be used to analyze N deficiency and to administer the N use efficiency in crop simulation model (Lemaire et al., 2008). The Nc is represented by an allometric function: Nc =  $aW^{-b}(1)$  where W is the total biomass expressed in t ha<sup>-1</sup>, Nc is the total N concentration in total biomass expressed in kg ha<sup>-1</sup>, and a and b are estimated parameters. The parameter *a* represents the N concentration when the total biomass is at least

It ha<sup>-1</sup> and the parameter b represents the coefficient of dilution which describes the relationship of decreasing N concentration with increasing total biomass. The critical N dilution curve defined by Eq. (1) is currently used to diagnose N deficiency, to manage N fertilization, and to simulate N uptake in crop models (Lemaire et al., 2008). It enables differentiation of three levels of the crop N status: (i) values significantly below the curve represent crop growth limited by N supply, (ii) values above the curve represent growth under luxury N supply, and (iii) values on the curve represent growth at Nc. Critical dilution curves for N have been determined for grasses (Marino et al., 2004), wheat (Justes et al., 1994), rice (Sheehy et al., 1998), oilseed rape (Colnenne et al., 1998), cotton (Xiaoping et al., 2007) and tomato (Tei et al., 2002). In potato, the values of the parameters a and b are estimated using the combined biomass of shoots and tubers, and the N concentration of this combined biomass. Greenwood et al. (1990), using data from Scotland and the Netherlands, reported values of a = 5.36 and b = 0.46 whereas Duchenne et al. (1997) in France obtained values of a = 5.21 and b = 0.56. Furthermore, the parameters were obtained under pedoclimatic conditions and with cultivars different from Tunisia. Our objectives were to determine the critical N curve for potato under the growing conditions and with cultivars widely grown under semi arid conditions, and to assess the possibility of using this critical N curve based on whole plants to estimate the level of N nutrition of potato grown under different N rates.

# Materials and methods

# Experimental design

Data were obtained from three field experiments conducted in Technical Center of Potato and Artichoke (CTPTA) (37°North, 10°South, Altitude 238 m) and Inter-professional Group of Vegetables (GIL) (36° 35' Nord, 10° 52'Est), in which we varied N applications, potato cultivars, sites, and years, as summarized in Table 1. In all the experiments, the cultivars were arranged in a completely randomized block design with three replications. N fertilizer was applied in three phases: vegetative growth, initiation of tuberization and maturation of tubers. The amount of P and K applied to satisfy plant growth demand were based on soil test recommendations. Further crop management procedures followed common agricultural practices to ensure maximum potential productivity.

# Sampling and measurement

Plants were collected using a 1m row section in each plot at different development stages (starting around 62 days after planting (DAP) until senescence in the first season, 44 DAP until senescence in the second season and 21 DAP until senescence in the third season). Shoot and tuber were weighed fresh and were collected for dry matter (DM) determination and laboratory analyses. DM was obtained by a forced-draft over drying at 75°C to constant weight. Dried samples were stored in plastic bags before laboratory analyses. The N concentration in shoots and tubers (N) was determined by kjeldahl method (Bremner, 1965) and was calculated by adding the N contents of shoots and tubers and dividing that by the total biomass. Total biomass (shoot and tuber biomass) and N concentration for each sampling date and year were subjected to analyses of variance using the STATISTIX9 (Analytical Software, 2014).

Basic information	2007/2008 season	2008/2009 season	2009/2010 season
Experiment	СТРТА	CTPTA	GIL
Soil characteristics (40 cm)			
Туре	Clay soil	Clay soil	Sandy soil
Organic matter (%)	2.5	2.4	1.4
pH	8.2	8.3	8.2
Total N (g kg <sup>-1</sup> )	1.2	1.4	0.8
$P(mg kg^{-1})$	50	74	79
K (mg kg <sup>-1</sup> )	767	880	343
Precipitation + Irrigation (mm)	419	340	370
Planting date	08/02/2008	02/03/2009	09/09/2009
Harvest date	01/06/2008	09/07/2009	18/01/2010
Cultivar			
	Spunta	Spunta	Spunta
		Bellini	Bellini
N rate (kg N ha <sup>-1</sup> )			
	0 (N0)	0 (N0)	0 (N0)
	50 (N50)	50 (N50)	50 (N50)
	200 (N200)	200 (N200)	200 (N200)
	300 (N300)		300 (N300)

Table 1. Basic information about three experiments conducted in CTPTA and GIL.

# Model calibration

Construction of the critical N dilution curve requires identification of critical data points at which N neither limits growth nor enhances it. An N limiting treatment was represented by treatments with significantly lower shoot and tuber biomass and in which supplementary N application led to a significant augment in DM. A non N limiting treatment was represented by treatments with significantly higher shoot and tuber biomass and in which N application led to an augment in N (Justes et al., 1994; Yao et al., 2014). In cases where more treatments was obtained with statistically the same total biomass, the treatment represented limiting N conditions when their N concentration was significantly lower and the treatment represented non limiting N when their N concentration was significantly higher (Giletto et al., 2012). On a chosen date of sampling, a critical N construction point was determined as follows: (i) each N treatment was characterized by its DM and N; (ii) the data for limiting N growth treatments were fitted by simple linear regression; (iii) the data for non limiting treatments were used to calculate the maximum DM from the averages of the observation data, (iv) the theoretical critical point was characterized by the calculated maximum DM; the N was the ordinate of maximum DM in a simple linear regression; and (v) fitting a power law regression equation to these theoretical critical points to determine the equation of the Nc curve (Justes et al., 1994). Data points were selected from non-N-limiting treatments (N200 and N300 treatments in 2007-2008 and 2009-2010 and N200 treatments in 2008-2009) for determination of the maximum N curve (Nmax); the data points from N limiting treatments (N0 and N50 treatments in 2007-2010) were used to construct the minimum N curve (Nmin).

Calculation of the nitrogen nutrition index (NNI) and the relative yield (RY) To characterize the N status of plants, the N nutrition index was used as follow: NNI = N/Nc (Eq. 2), where N is the total N concentration measured in shoots and tubers, and Nc is the

critical N concentration for the same biomass. The presented results of NNI are restricted to N0, N50 and N200 treatments. The relative yield (RY) was calculated as the ratio of the final harvest tuber yield obtained at a given rate of N application and the highest tuber yield among N rates (Zhao, 2014). RY was expressed as a function of NNI and the linear function was estimated using STATISTIX9 (Analytical Software, 2014). The presented results of RY are restricted to N0, N50 and N200 treatments.

# **Results and discussion**

# Establishment of the critical N dilution curve

Data points for each sampling date from vegetative growth to maturation of tubers for both cultivars were used to determine the Nc points by following the computation method of Justes et al. (1994). Forty four data points between 0.36 and 9.70t ha<sup>-1</sup> of DM allowed us to calculate Nc. The Nc points were determined by intercept between the vertical and oblique lines fitted through the data points on each sampling date for Spunta and Bellini, respectively (Fig. 1). There was a declining trend of Nc values in both cultivars with increasing DM, with determination coefficients of 0.99 and 0.93 for Spunta and Bellini, respectively (Fig. 2). This phenomenon, has to be considered as an ontogenetic process (Lemaire and Gastal, 2009) and is based on the premise that plant DM comprises two compartments: DMm, the dry matter of metabolic tissues involved directly in plant growth process (photosynthesis and meristematic activity), which has a high N concentration, and DMs, the structural tissues in plant architecture that have a low N concentration. During the growth process, the proportion of DMs is large, and the proportion of DMm is small; accordingly, the N concentration of the plant declines with growth (Lemaire et al., 2008). The trend lines were fitted as follows:  $0.36t ha^{-1}$ ,  $R^2 = 0.99$ ) (Eq. 3), Bellini: Nc =  $2.99W^{-0.38}$  (W Spunta:  $Nc = 3.25W^{-0.31}$  (W  $0.36t ha^{-1}$ ,  $R^2 = 0.93$ ) (Eq. 4).

The trends in Nc curves were consistent between the two cultivars; a negative power function fit both. The two curves were not significantly different (p > 0.05) according to calculation procedures recommended by Hahn (1997). Hence, the data for two varietal groups were pooled together and a unique dilution curve was fitted as follows (Eq. 5): Nc =  $3.18W^{-0.37}$  (W 0.36t ha<sup>-1</sup>, R<sup>2</sup> = 0.87) (Eq. 5).

The parameters *a* of 3.18% and *b* (0.37) in this work were lower than those reported by Giletto et al. (2012) (a = 5.30%, b = 0.42), Bélanger et al. (2001) (a = 4.37%, b = 0.50), Duchenne et al. (1997) (a = 5.21%, b = 0.56) and Greenwood et al. (1990) (a = 5.36%, b = 0.46), because the sampling period, climatic and edaphic conditions were different to these authors. Parameter *b* in our work (0.37) was higher than the theoretical value of 0.34 defined by Greenwood et al. (1990) based on the principle that there is a strong link between N in fescue (*Festuca arundinacea* L.), alfalfa (*Medicago sativa* L.) and wheat and its metabolic activities. When considering only the potato data, Greenwood et al. (1990) explained this difference by the presence in potato of significant quantities of N in tubers. According to Yao et al. (2014), the N dilution levels of Nc of fescue, alfalfa and wheat were less marked than that of the Nc of potato.

**Figure 1.** Critical N data points used to define Nc curves (a: 2007-2010 Spunta, b: 2007-2010 Bellini). The symbols ( $\diamondsuit$ ) and () represent the values measured in the N-limiting treatments for Spunta and Bellini, while (+) and (x) represent the values measured in the non N limiting treatments for Spunta and Bellini. The symbol ( $\bullet$ ) represents the calculated Nc points for each date. The solid line represents Nc curve describing the relationship between Nc and DM of potato.

The model accounted for 87% of the total variance. The 95% confidence interval of the mean was 3.30% DM for a shoot and tuber biomass of 0.36t ha<sup>-1</sup>, while 3.10% DM for a shoot and tuber biomass of 9.70t ha<sup>-1</sup> (Fig. 3). The Nc dilution curve cannot be applied to low DM (< 0.36t ha<sup>-1</sup>) due to relatively smaller decline of Nc with increasing DM during early growth stages, when plants were spatially isolated. Therefore, 18 data points, ranging from 0.36 to 0.76t ha<sup>-1</sup>, were used to determine the constant Nc at low DM (< 0.36t ha<sup>-1</sup>). The constant Nc was calculated as the mean value between the minimum N concentration of non limiting N points (4.51% DM) and the maximum N concentration of limiting N points (3.14% DM). The constant Nc value used here was 3.84% DM. The Nc curve and the constant Nc concentration at low DM intersected at DM value of 0.52t ha<sup>-1</sup>; so, for a shoot and tuber biomass < 0.52t ha<sup>-1</sup>, the critical shoot and tuber N concentration best fitted with a constant value of 3.84% DM. On the other hand, for shoot and tuber biomass > 0.52t ha<sup>-1</sup>, the critical N dilution curve describes the critical N concentration.

### Validation of the Nc dilution curve

The critical curve was validated both for limiting (Fig. 3-a) and non-limiting (Fig. 3-b) situations within the biomass range for which it was established. Results indicated that growth rate and cultivar did not significantly affect Nc. The wide range of pedo-climatic conditions included in our dataset (semi arid conditions of Tunisia) suggests that the potato Nc dilution curve was also independent of the growing environments. As seen in Fig. 3-a and 3-b, all the data points from the N limiting treatments were close to or below the Nc dilution curve. Whereas, those from the non N limiting treatments were close to or above the Nc dilution curve. The Nmax curve represents the maximum N accumulation capacity of the plant (luxury consumption of N); while the Nmin curve represents the lower limit at which the metabolism would cease to function (Giletto et al., 2012). The variability of N in the soil. Between the Nc and the Nmax, N absorption is determined by high mineral N availability in the soil and is independent of the growth rate. Between the Nc and the Nmin curve, N absorption is limited by low mineral N availability in the soil and determines the growth rate (Justes et al., 1994).

Variability of NNI with development stage under unterent is rates

The Nc dilution curve allows an accurate diagnosis of potato N nutrition status (Ata-Ul-Karim et al., 2013). To characterize the N status of plants, the NNI was calculated by using Eq. 2. If NNI = 1, N nutrition is considered as optimum, while NNI>1 indicates excess N and NNI<1 indicates N deficiency. Therefore, NNI can be used to quantify the degree of N stress. Significant differences were observed for NNI across the treatments and cultivars at different sampling dates. The NNI ranged from 0.42 (N0) to 1.03 (N200) for Spunta and 0.49 (N0) to 1.04 (N200) for Bellini during 2008-2009, while 0.73 (N0) to 1.22 (N200) for Spunta and 0.49 (N0) to 1.086 (N50) to 1.22 (N50) for Bellini during 2009-2010 (Fig. 4). The NNI values for treatment N200 in the 2008-2009 and 2009-2010 seasons were to 1, indicating that N levels were optimal (non N limiting treatment). The values of NNI were < 1 for treatments N0 and N50 in 2008-2010 seasons (N limiting treatments). In 2009-2010, the NNI increased in N0 and N50 at the beginning and the end of the growing cycle, probably due to N mineralization from the soil organic matter (Fig. 4).

These results confirm the robustness of NNI as a measure of shoot and tuber N status in potato under semi-arid conditions.

Relationship between the Relative Yield (RY) and Nitrogen Nutrition Index Figure 5 shows the relationship between RY measured at harvest and NNI measured at 89 DAP in 2008-09 and at 66 DAP in 2009-10. The relation between them was expressed by a linear function and accounted for 51% and 66% of the variation for Spunta and Bellini, respectively. The relationship between RY and NNI of Spunta and Bellini appear to be similar for Shepody ( $R^2 = 82\%$ ) and Russet Burbank ( $R^2 = 71\%$ ) under irrigated conditions (Bélanger at al., 2001) and for Innovator ( $R^2 = 69\%$ ) (Giletto et al., 2012). For a NNI greater than 1.0, the relative yield was near 99% and 92% for Spunta and Bellini, respectively (Fig. 5). With decreasing NNI, below 1, the relative yield decreased. **Figure 4**. Changes of nitrogen nutrition index (NNI) with time (Days of plantation) for potato cultivars (Spunta and Bellini) under different N application rates in experiments conducted during 2008-2009 (a) and 2009-2010 (b).

Figure 5. The relationship between relative yield and the N nutrition index of Spunta and Bellini.

### Conclusion

A critical N dilution curve (Nc =  $3.18W^{-0.37}$ ) was developed for Spunta and Bellini in northest Tunisia. This curve was different from those developed for potato in Canada, France and Argentina. The resulting NNI was calculated from this critical N dilution curve and was highly related to relative yield. Therefore, the concept of a critical N concentration provides a reference method for assessing the status of N nutrition during crop growth in Northest Tunisia

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