Seed Germination and Early Root Growth in Common Bean and Maize Landraces and Improved Cultivars at Different Water Stress Levels

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Abstract

In the Central Valleys region of Oaxaca, Mexico, around 90 % of the area dedicated to common bean and maize production is planted with landraces under rainfed conditions. The objective of this study was to assess germination and early root growth of maize and common bean landraces and improved cultivars with moisture deficit. Using a completely random design and factorial treatment array, four cultivars of each species and three osmotic potentials (Ψo: 0, -0.5 and -1.1 MPa) were assessed. Maximum accumulated germination time was significantly increased ($P \leq 0.05$) with Ψo = -1.1 MPa, mainly in improved cultivars. Maximum accumulated germination with -1.1 MPa decreased ($P \leq 0.05$) more drastically in improved cultivars. Root length and biomass decreased up to 80 and 70 %, respectively, in improved cultivars. Germination of maize and bean landraces was less affected by restricted moisture in the germination medium than improved cultivars.

Keywords: *Zea mays* L., *Phaseolus vulgaris* L., staple crops, water potential

Introduction

Maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) are the most important crops of the agricultural sector of Mexico, as well as being elements of cultural identity (Secretaría de Economía, 2012; Guzmán and León, 2011). Both species are cultivated in a broad variety of environments, in terms of altitude, temperature, soil type, and moisture regime (Hellin *et al*., 2013; Peña-Betancourt and Conde Martínez, 2012). But they are grown mainly in dryland and rainfed conditions. Water availability, therefore, with intermittent periods of drought at any moment of their growing cycle is the main factor limiting yields (Barrios-Gómez *et al*., 2010; Rivera-Hernández *et al*., 2010).

The Central Valleys region of Oaxaca, having vast territory with BSh type (hot semi-arid) climate, is first and second in area cultivated under maize and beans in the state. It is well known that local farmers have selected landraces collections that best adapt to the soil and climate conditions of the region (Pliego-Marín *et al*., 2013). Although maize and bean are the basis of food security in this region (Badstue *et al*., 2007), they are grown in a rainfed regime (SIAP-SAGARPA, 2012). Thus, scarce soil moisture is one of the factors related to low yields in the maize-bean association system (Ruiz and Loaeza, 2004).

Genetic improvement is considered an option for stabilizing and increasing yields of cultivars grown with limited moisture (Rosales-Serna *et al*., 2000; Cinta *et al*., 2010).

However, data from SIAP-SAGARPA (2011) show that only 10 % of the maize and bean seed used in the Central Valleys of Oaxaca are hybrids and improved varieties developed basically for level areas with irrigation or sufficient rain, even though public institutions such as Universidad Autónoma Chapingo and the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) have released at least six hybrids and 11 improved landraces varieties of maize. For beans, a commercial variety (Negro Michigan) and the varieties Negro Jamapa and Negro Tacaná are available. The latter is considered one of the most productive varieties for cultivation in dry conditions (López-Salinas *et al*., 2011).

Some specialists have pointed out that the impact of public institutions on seed commerce is still limited even though the existence of outstanding genetic materials has been documented. This is attributed in part to the deficient multiplication scheme and supply of registered seed (Luna *et al*., 2012), as well as to the fact that improvement has focused more on obtaining agronomically superior varieties while neglecting quality characteristics such as cooking time and flavor (Acosta-Gallegos *et al*., 2000).

Farmers resist adopting improved seed mainly because they have observed that commercial improved seed does not adapt to their agroecological conditions and its use increases requirements of fertilizers, pesticides and water, thus raising production costs and limiting their profits. Moreover, landraces varieties have characteristics that make them particularly apt for use in the preparation of specific dishes (for example, tlayuda, Oaxacan totopo, tamales, nicuatole and tejate) and in cultural practices (Turrent, 2012).

Soil moisture deficit conditions negatively affect germination, seedling establishment and initial growth. These reduce plant density and also, invariably, yield per unit of area (Teruel *et al*., 2008).

Germination is one of the most important stages in the life of plants that determine the efficient use of the nutrients and water resources available to plants. Edmeades *et al*. (1994) demonstrated that a prolonged period of moisture deficit during establishment of a maize crop can cause a decrease in yield, comparable to one caused by drought during flowering. Genotype-dependent response has also been documented (Pérez *et al*., 2007).

Common bean is a species of recognized intolerance to moisture deficit (Acosta-Díaz *et al*., 2004) and the germination stage is one of the most sensitive (Cokkizgin, 2012). However, it has also been recognized that landraces cultivars show moderate tolerance to moisture deficit (Fernández-Rivera *et al*., 2007).

Seed germination tests under controlled conditions and with a factor that induces physiological tension, such as moisture deficit, are conducted to determine seed quality and also to contrast tolerance to moisture deficit between cultivars or species (Peña-Valdivia *et al*., 2010; Tsougkrianis et al. 2009). Some of these tests use high molecular weight polyethylene glycol (PEG) to modify moisture availability in the germination medium, since it has been demonstrated that the germination rate in PEG is similar to that in soil (Gharoobi *et al*., 2012).

The objective of this study was to assess germination and initial root growth in maize and bean in function of germination medium moisture and of cultivar for each species separately. The proposed hypothesis was that landraces are more successful than hybrids in conditions of water deficit.

Materials and Methods

Seed from three maize landraces of the blanco bolita race (BB1, BB2, BB3) and landraces thin black beans (ND1, ND2, ND3), collected in Villa de Zaachila, Central Valleys, Oaxaca Mexico (16º 56´ latitude N, 96º 45 longitude W and 1520 masl) were used. The seed was produced in a rainfed regime during the spring-summer cycle, 2011. Improved bean cultivar Tacaná (Tac) donated by INIFAP and the maize hybrid H-375 recommended for the region by the Centro Regional del Sur de la Universidad Autónoma Chapingo (UACh) were also included. The seeds were kept in isolation and refrigeration (4±1ºC) for 60 days until assessment.

The assessments were conducted in the Environmental Biophysical and Plant Physiology Laboratory of Plant Biophysics and Plant Physiology of the graduate program in Botany of the Colegio de Postgraduados, Campus Montecillo, México.

Landraces germplasm was identified by cooperating farmers as cultivars planted continuously in the region for at least 30 years. Plant morphological characteristics and reproductive structures of each species were compared in the field. The general characteristics assessed in the laboratory for description of germplasm were 100 seed weight and moisture. The range for 100 seed weight was 35.4 to 49.2 g for maizes and 14.1 to 16.5 g for beans, with statistical differences that are discussed in the results. There were no statistical differences for moisture (in a sample of 20 seeds with four replications); values were from 8.9 to 9.8 % in maizes and 15.2 a 16.5 % in beans.

With the standard "between paper" germination test (25 seeds and four replications) recommended by ISTA (2005) values of 85 to 94 % germination of maizes and 88 to 94 % germination of beans were obtained, with no statistical differences.

For each crop, a completely randomized experimental design was used with a 4 x 3 factorial array and four replications. The factors and levels were cultivars (three landraces and one improved) and osmotic potential (three Ψo; 0.0, -0.5 and -1.1 MPa); the experimental unit was 15 seeds.

Germination with limited moisture was tested in Petri dishes (125 mm diameter) with filter paper moistened with 10 mL distilled water (control) or aqueous solutions of polyethylene glycol (PEG 8000; Sigma-Aldrich) with Ψo = -0.5 y -1.1 MPa. These values were verified with a vapor pressure osmometer (Wescor 5520, Wescor, Inc., Logan, UT, USA) at 25 ºC. Seeds were disinfected by shaking in an aqueous solution of 5 %, sodium hypochlorite (NaClO 5 % active chlorine) for 15 min; excess hypochlorite was eliminated with distilled water.
The seeds in Petri dishes were kept in a germinator at 25 ± 1 ºC in darkness for 148 h. Petri dishes were weighed every 48 h, and evaporated water was replaced. Every 12 h germinated seeds (root ≥ 5 mm long) were quantified and kept in the Petri dish. In this way, the time it took to reach 50 % of the maximum germination (T50), maximum accumulated germination (when no more seeds germinated), the number of abnormal seedlings (without root but with other exposed structures) and ungerminated seeds were recorded. Root length was measured with a digital caliper (Caliper-Mitutoyo, Japan) and precision of ± 0.0001". The roots were harvested, washed with distilled water and dehydrated at 70 ºC. Biomass was weighed with an analytical balance (Scientech (SA100, USA) with 0.0001 g precision.

The data obtained were analyzed with the Shapiro Wilk Test and variables were transformed with the arcsine square root when the assumption was not met. The analysis of variance was performed with SAS, version 9, and statistical software. The Tukey test ($P \leq 0.05$) was used for multiple comparisons of means. The figures and fit models for germination curves were processed with SigmaPlot software of Jandel Scientific (Version 10).

Results and Discussion

In the two crops T50 increased ($P \leq 0.05$) with the decrease in available moisture. Moreover, the responses were dependent on the crop and on the cultivar (Figure 1). The mean T50 of maize landraces controls (Ψo=0.0 MPa) was 24 h, while that of H-375 was 42 h. The delay in germination with Ψo=-0.5 MPa was almost double that of the landraces cultivars (45 h on average) and of the improved cultivar (70 h). The decrease in T50 caused by Ψo=-1.1 MPa was less evident than in the improved cultivar; in the former the values were 54 to 60 h and in the latter it reached 95 h (Figure 1 E).The T50 of the four bean controls varied little (16 a 20 h); in ND1, ND2 and Tacaná there was no change relative to their controls with -0.5 MPa. In contrast, ND3 was the only treatment in which T50 decreased significantly. With higher moisture restriction (-1.1 MPa), the four cultivars reached T50 between 36 and 42 h (Figure 1 F).

The results confirmed that germination speed of maize and common bean seed in conditions of limited moisture is dependent on the cultivar (Aguilar, 2011; Tsougkrianis *et al*., 2009). This may be due to differences in permeability of the testa and other seed structures such as cotyledons (Dübbern De Souza and Marcos–Fihlo, 2001) and to the seed chemical composition (Meyer *et al*., 2007).

Maximum accumulated germination (MAG) of the eight cultivars, with no moisture restriction, was on average between 78 and 82 % (Figure 1 A-B), while with limited moisture ($P \leq 0.05$) MAG decreased to values between 48 and 56 % in both crops (Figure 1 E-F). The reduction in bean germination was cultivar-dependent. In contrast, for maize no statistically significant interaction was found (Table 1).

MAG of the two crops decreased significantly with the lowest Ψo. In maize the Ψo that significantly affected cv. BB3 MAG was -0.5 MPa, although the decrease was only 14 % lower than the condition without moisture restriction. In contrast, the highest moisture restriction (Ψo = -1.1 MPa) decreased MAG of the landraces by 20 and 25 % and by more than 30 % that of the improved cultivar (Figure 1 E).

In bean cultivars, partial moisture restriction (-0.5 MPa) decreased MAG of landraces and of the improved cultivar by 5 to 18 % (Figure 1 D). With the highest moisture restriction (-1.1 MPa) MAG decreased notably in landraces cultivars, from 20 % in ND3 and up to 50 % in ND1. The MAG of the improved cultivar, however, fell nearly 80 % (Figure 1 F).

Other studies have shown that MAG in beans with water potentials of -2.0 MPa decreases more drastically than that of maize. This response has been attributed to the fact that bean seed can imbibe moisture equivalent to up to 90 % of its mass, while maize imbibes only 40 % (Aguilar, 2011). Thus, it can be inferred that with lower availability of water, bean seed cannot imbibe enough moisture to germinate, as compared with maize seed, which does.

The main factors, i.e. cultivar (C) and osmotic potential (Ψo), as well as the interaction cultivar x osmotic potential (C x Ψo) showed significance in most of the assessed variables (Table 1). For both crops the interaction (C x Ψo) was highly significant ($P \leq 0.01$) in percentage of ungerminated seeds. This was also manifested in maize root length and root biomass and in maximum accumulated bean germination as well as in contaminated seeds.

Maximum accumulated germination of maize correlated positively with seed weight (r = 0.97 **, Table 2). Mean maize seed weight was different ($P \leq 0.05$) among all cultivars; the lowest value was that of H-375 (35.4 g/100 seeds) and the highest was that of BB3 (49.2 g/100 seeds). There is a broad diversity of maize seed in terms of size, shape and composition due to genetic and environmental factors and to the location of the caryopsis on the ear (Pérez *et al*., 2007). Also, seed weight is the result of adaptation of the mother plant to climate changes in its ecological niche, giving it greater stability (Pérez *et al*., 2007). This may partially explain why landraces cultivars had the heaviest seed and the highest percentages of germination in the condition of water stress.

Soltani *et al*. (2002) documented that large seeds have a higher percentage of germination and require less time to germinate. The advantages of large seeds can be associated with embryo size and their capacity to provide more energy. These authors pointed out that with low osmotic potential the rate of utilization of reserves in the plant's tissues decreases progressively. This affirmation coincides with what we observed in maize cultivars in our study (Table 2).

In beans, the correlations between weight and maximum accumulated germination were not significant (Table 2). The cultivar ND3 weighed less (14.1 g/100 seeds) than the other three cultivars (15.5 to 16.5 g/100 seeds), but there were no significant differences ($P > 0.05$). Seed weight of this crop is considered a trait closely linked to duration of the reproductive period (Martínez *et al*., 2007).

There was a positive correlation between root length and root biomass in both crops (Figure 2). In maize, a significant interaction between cultivar and Ψo was identified, indicating that root growth is dependent on both species and the moisture condition of the germination medium, while in beans there was a significant effect of Ψo but not of the cultivar (Table 1).

Cultivar H-375 was 22 % shorter and had 62 % less biomass, relative to the landraces cultivars, among which there were no significant differences. The lowest Ψo, however, diminished root length and biomass by up to 55 and 91 %, respectively (Figure 2). Peña-Valdivia *et al*. (2007) proved that, with a substrate water potential (Ψw) of -1.58 MPa, maize root length, fresh matter and dry biomass decreased by a half or less, relative to values obtained with Ψw of -0.03 MPa, indicating that the crops used the limited moisture of the substrate efficiently to germinate, but they did not achieve adequate growth of the radical system. The initial root growth is a process driven by turgidity of the cells of the embryonic axis (Bewley, 1997), which is largely regulated by moisture content in the growth medium. Our results showed advantages to the maize landraces over the hybrid assessed, in terms of a higher probability of survival and seedling establishment under moderate conditions of water stress.

The maize cultivars with heavier seed (BB1 and BB3, 45.6 and 49.2 g/100 seeds, respectively) produced larger and heavier roots. In contrast, the bean cultivars assessed were different in seed weight but did not exhibit differences ($P \geq 0.05$) in initial root growth.

These findings do not agree with the observations of Allende-Arrarás *et al*. (2006), these researchers found a positive association between common bean seed size and vegetative development. In this respect, Celis-Velázquez *et al*. (2008) reported that the relationship between seed weight and vigor depends on the race. In our study, the Ψo of -1.1 MPa reduced root length and root biomass of seedling beans up to 81 and 73 %, respectively, regardless of seed size (Table 1). Previous studies have shown that biomass in some cultivars may not be affected by seed size, and it is speculated that imposed water stress, regardless of seed size, reduces the percentage of reserves mobilized for initial growth (Gholami *et al*., 2009).

Landraces were less affected by more negative Ψo. Nevertheless, despite disinfecting the seeds previously to the study, it was observed that in Ψo of -0.5 and -1.1 MPa there was a larger number of landraces seeds were contaminated by fungi than hybrid seeds (Table 1). This effect had been observed previously, and it is known that vegetative development of some pathogenic fungi is favored by water deficit; they grow vegetatively and infect their hosts more severely (Lira and Mayek, 2006). In this respect, it has been documented that the farmers of the Central Valleys of Oaxaca maintain a flow of seed from farmer to farmer, with flexible processes of selection with which they have obtained cultivars adapted to heterogeneous environments. However, this process is based mainly on visible seed traits, and thus factors such as pathogens or inappropriate storage may go undetected (Badstue, 2007) and persist in the selected cultivars.

We hope that results of this study contribute to strengthen arguments on the agroecological value of landraces maize and bean cultivars in the Central Valleys of Oaxaca. It is still necessary to find options for improving sanitary management during seed multiplication to increase efficiency in seedling establishment and to document the attributes of outstanding cultivars on which to base their management.

Conclusions

Germination and initial growth of maize and bean roots decreased with Ψo = -1.1 MPa.

Germination and initial root growth of landraces cultivars responded better to water stress than improved cultivars.

In maize, germination and initial root growth were affected by cultivar and Ψo value, although, in most, additive effects were seen. Also, significant interactions were observed, mainly in the initial root growth variables.
In beans the variables with significant interactions were related to germination, while initial growth depended largely on the Ψo condition.

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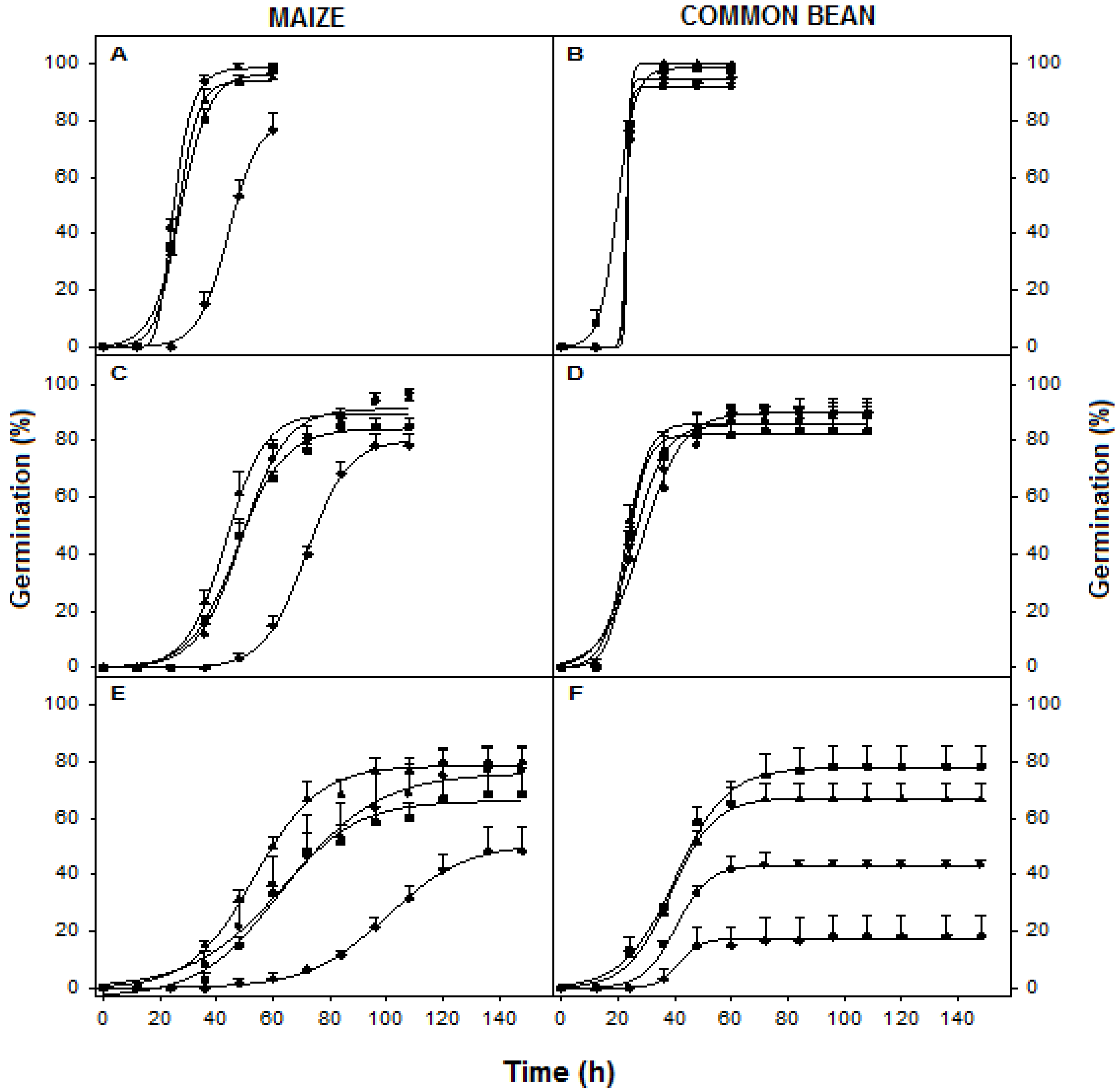

Figure 1: Accumulated germination of three landraces maize and common bean landraces (BB1-maize and ND1-bean = circles; BB2-maize and ND2-bean = triangles; BB3-maíze and ND3-bean = squares) and improved (H-375-maize and Tac-bean = rhomboids) at three osmotic potentials (-0.0 MPa, A-B; -0.5 MPa, C-D and -1.1 MPa, E-F). n = 4 + standard error

Table 1: Germination and maize and common bean seedling characteristics on an osmotic potential (ψo) gradient

Factor	Seeds (%)			Seedlings (%)		Root (mm)	Root (g)
	Ungerminated	Contaminated	Germinated	Normal	Abnormal	Length	Biomass
MAIZE							
Cultivar							
C M1	6.96 a	4.98 b	77.03 a	74.21 a	8.65 b	53.26 a	0.075 a
C M2	0.00 c	19.05 a	73.78 a	66.04 a	10.86 ab	50.56 ab	0.080 a
C M3	0.00 c	17.69 a	70.11 a	65.50 a	13.75 ab	54.10 a	0.074 a
H-375	18.67 a	3.03 b	56.01 b	54,52 b	19.75 b	41.93 b	0.030 b
Significance	**	*	**	**	**	*	**
Ψo (MPa)							
0.0	0.00 b	5.07 b	78.20 a	79.17 a	4.14 b	70.44 a	0.11 a
-0.5	4.21 b	10.88 b	72.86 a	66.81 b	14.53 a	47.72 b	0.05 b
-1.1	15.01 a	17.62 a	56.64 b	49.22 c	21.09 a	31.73 c	0.01 c
Significance	**	*	**	**	**	**	**
C x Ψo	**	NS	NS	NS	NS	*	*
COMMON BEAN							
Cultivar							
C F1	0.00 b	29.79 a	61.85 b	54.43 b	14.04 a	23.03 a	0.028 a
C F2	8.39 b	9.33 b	73.51 a	66.08 a	11.01 a	29.98 a	0.034 a
C F3	5.96 b	11.84 b	72.19 a	66.72 a	9.55 a	28.02 a	0.034 a
Tac	23.44 a	6.15 b	58.27 b	49.85 b	8.29 a	29.29 a	0.024 a
Significance	**	**	**	**	NS	NS	NS
Ψo (MPa)							
0.0	0.00 b	5.48 b	82.10 a	79.96 a	0.00 a	46.13 a	0.037 a
-0.5	3.81 b	17.21 a	71.35 b	64.00 b	9.13 b	28.07 b	0.043 a
-1.1	24.53 a	20.15 a	47.91 c	33.85 c	23.04 c	8.54 c	0.01 b
Significance	**	*	*	**	**	**	**
C x Ψo	**	*	*	NS	NS	NS	NS

Values with the same letter in each column are statistically equal (Tukey P≤0.05). Means calculated with transformed data. NS: not significant.

Table 2: Pearson bivariate correlations between seed weight and germination

	SW	MAG	SW	MAG
	MAIZE		COMMON BEAN	
SW	1.00		1.00	-----
MAG	0.97*	1.00		1.00

SW = seed weight, MAG = maximum accumulated germination * = P ≤ 0.05.

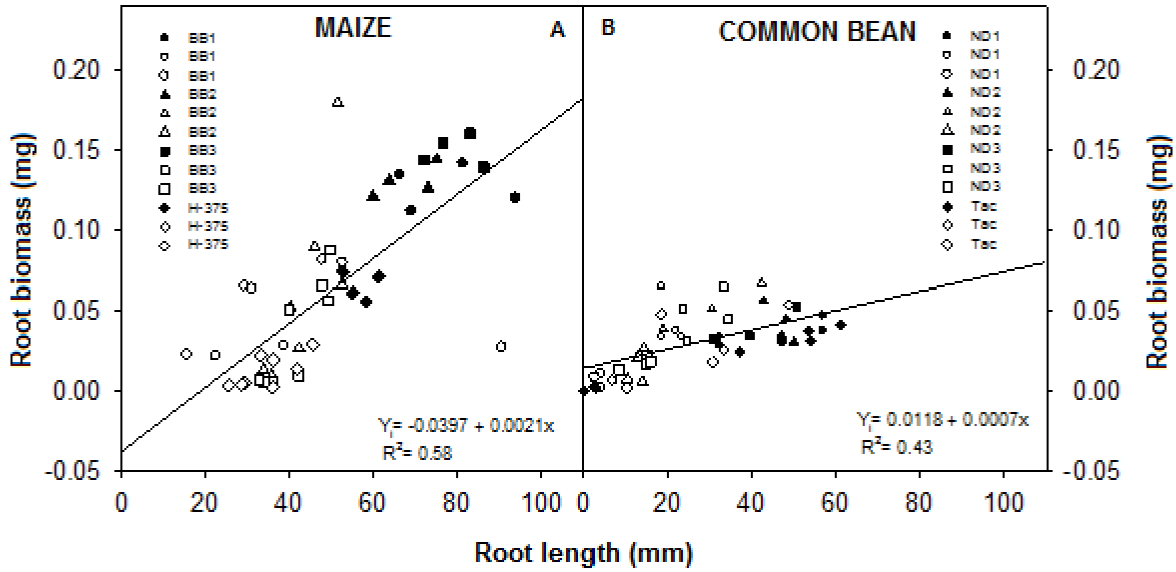

Figure 2: Initial root growth in four maize and common bean cultivars under three conditions of osmotic potential (0 –shaded symbols-, -0.5 –white symbols- and -1.1 MPa –black symbols-)