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Morphological Characterization of NaCl-tolerant *Phaseolus vulgaris* Seeds

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ABSTRACT

Bean is a source of proteins but salinity is an agricultural limiting factor. Evaluation of the plant germplasm to identify a tolerant genotype is an adequate strategy. The present report is focused on the early stage of bean seed germination. Seeds of 89 cultivars were weighed after 5 days of growth in 0 or 270 mmol/L NaCl. The five heaviest cultivars were regarded as NaCl tolerant; and the five lighter, as susceptible. Ten fresh seeds per cultivar were scanned. The following indicators were measured: area, equivalent diameter, major axis length, minor axis length, eccentricity, convex area, solidity, and extent. The matrix obtained was used to estimate Fisher's linear discriminant functions for susceptible and tolerant cultivars. Two variables were disregarded (convex area and solidity). Functions classified correctly 100% of tolerant or susceptible originally grouped cultivars. Fisher's linear discriminant functions are important tools for bean breeders. Seeds from new bean genotypes can be scanned as described here. Data were evaluated in both discriminant functions. If the resulting value of the tolerant-discriminant function was statistically higher than that of the susceptible-discriminant function, then the new bean genotype can be regarded as putatively tolerant. In addition to the practical use of the assay described here, we reached two unexpected conclusions. Firstly, bean seeds were not so NaCl-sensitive during early stages of germination: we observed radical emergence with a high concentration (360 mmol/L). Secondly, the larger the bean seed size, the lower the sensitivity to NaCl.

Keywords: area, axis length, bean, convex area, diameter, eccentricity, equivalent solidity, extent, Fisher's linear discriminant functions, MATLAB, salinity

INTRODUCTION

Common bean is an important source of proteins in developing countries (Durante and Gius 1997). It is sensitive to salts, like many other leguminous crops (Maas and Hoffman 1977). Soil salinity is an increasingly severe agricultural problem in several world regions (Shannon 1986), e.g. in a narrow island such as Cuba. The evaluation of genetic variability of plant germplasm to identify a tolerant genotype has been one of the most frequent strategies to overcome this problem (Kingsbury and Epstein 1984).

Characters such as yield, survival, vigour, leaf damage, and plant height, have been the most commonly used criteria for identifying salinity tolerance (Maas and Hoffman 1977; Shannon 1984). Other indices of tolerance have been proposed that are based on specific physiological characteristics, for instance, accumulation of specific ions in shoots or leaves, or the production of a specific metabolite (Shannon 1986).

Salinity tolerance, however, is usually assayed in terms of absolute or relative growth or yield (Maas and Hoffman 1977; Shannon 1984). This is largely due to ease of measurement and because, in the end, yield (both absolute and relative) under saline conditions is usually the ultimate goal (Bayuelo-Jiménez *et al.* 2002).

Evaluation of salt tolerance in legumes has been attempted by a variety of cultural techniques with plant material ranging from germinating seeds to seedlings to mature plants (Prisco and O'Leary 1972; Keating and Fisher 1985; Subbarao *et al.* 1991; Ortiz *et al.* 1994; Ferri *et al.* 2000; Lopez *et al.* 2002; Jungklang *et al.* 2003; Aroca *et al.* 2007; Murillo-Amador *et al.* 2007; Webber *et al.* 2008; Rogers *et al.* 2009; Priyanka *et al.* 2010). Evidence collected from various species suggests that salt tolerance is a developmentally regulated, stage-specific phenomenon, so that tolerance at one stage of development may not be correlated with tolerance at other developmental stages (Shannon 1986; Bayuelo-Jiménez *et al*. 2002).

The present report is only focused on the early stage of bean seed germination. It describes the generation of Fisher's linear discriminant functions for morphological identification of NaCl-tolerant bean seeds. To our knowledge, the relationship between the bean seed size and its tolerance to sodium chloride has not been published to date.

MATERIALS AND METHODS

Seeds of 89 bean cultivars were provided by the Cuban National Institute for Agricultural Sciences (INCA, Havana, Cuba). One of these cultivars was randomly selected to test the effect of sodium chloride on seed germination. Petri dishes (ø: 15 cm, height: 2.5 cm) with filter papers (Whatman, single layer) were used. Five concentrations dissolved in distilled water were compared (0, 90, 180, 270, 360 mmol/L). Each dish contained 10 seeds and 15 mL solution. Fresh plantlet mass was recorded after 5 days of incubation in the dark. In a second experiment, the effect of two concentrations of NaCl (0, 270 mmol/L) on the whole bean collection was evaluated as described above.

The five heaviest cultivars were regarded as NaCl-tolerant; and the five lighter, as susceptible. Ten fresh seeds per cultivar were simultaneously scanned (jpg format, 600 dpi color image, Cannon F189200 ImageRunner 1023N, Cannon IR Toolbox 4.9). The following eight indicators were measured from the scanned image by MATLAB (version 7.01.247014 (R14), Service pack 1; September 13, 2004): area, equivalent diameter, major axis length, minor axis length, eccentricity, convex area, solidity, and extent

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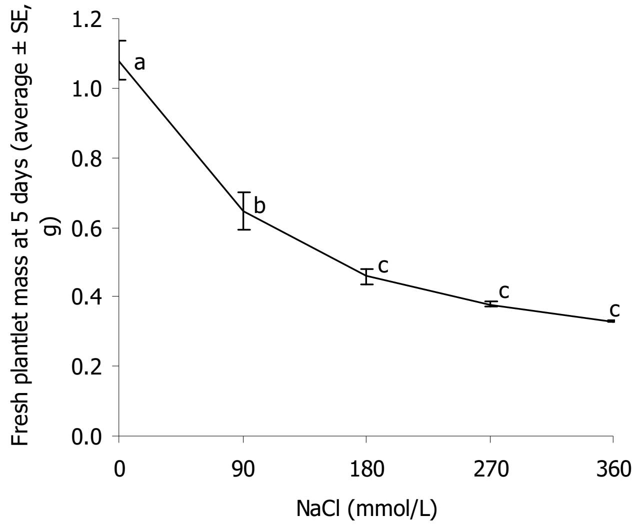

Fig. 1 Effect of sodium chloride on bean seed germination.

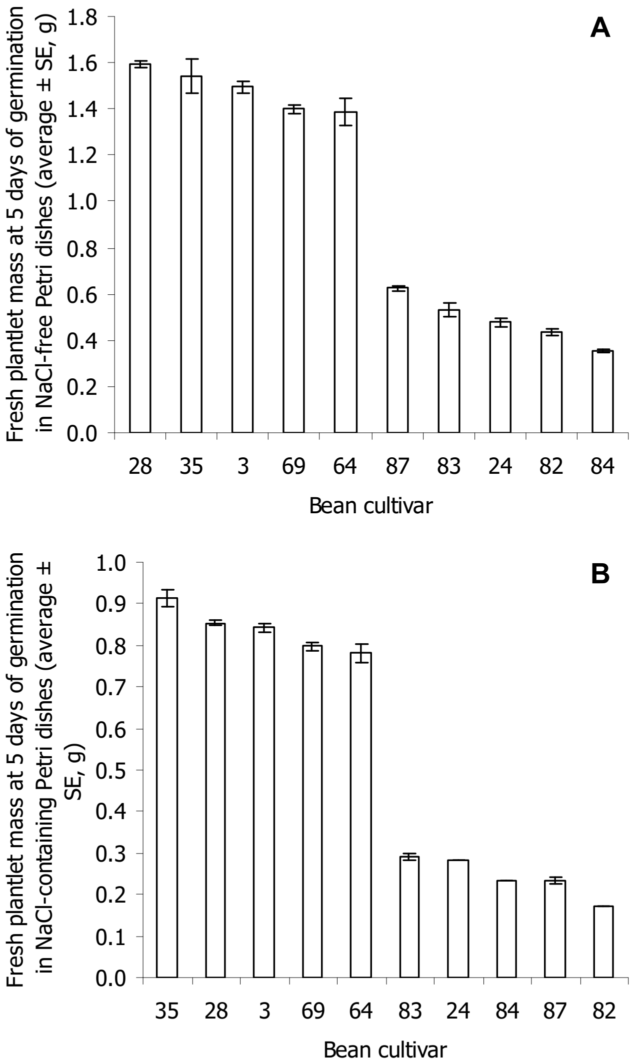

Fig. 2 Been seed germination in sodium chloride-free environment or with 270 mmol/L.

(Mathlab help version 7.01.247014 (R14), Image Processing Toolbox User's Guide, regionprops functions).

Area (scalar) is the actual number of pixels in the region. Equivalent diameter (scalar) is the diameter of a circle with the same area as the region. Major axis length (scalar) is the length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region. Minor axis length (scalar) is the length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.

Eccentricity (scalar) is related to the eccentricity of the ellipse that has the same second-moments as the region. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length.

Convex area (scalar) is the number of pixels in convex image. The convex image is a binary image (logical) of the convex hull. The convex hull is the smallest convex polygon that can contain the region.

Solidity (scalar) is the proportion of the pixels in the convex hull that are also in the region (computed as area/convex area). Extent (scalar) is the proportion of the pixels in the bounding box that are also in the region (computed as the area divided by the area of the bounding box).

The matrix obtained (10 bean cultivars, 10 seeds/cultivar, 8 indicators) was standardized according to Kantardzic (2003). Later, the matrix was used to estimate Fisher's linear discriminant functions for susceptible and tolerant cultivars. The Statistical Package for Social Sciences (Version 8.0 for Windows, SPSS Inc.) was used.

RESULTS AND DISCUSSION

NaCl reduced seed germination (**Fig. 1**). **Fig. 2** shows the results of the most contrasting members of the germplasm collection. Cultivars 28, 35, 3, 69 and 64 germinated faster than 87, 83, 24, 82 and 84, in both NaCl-free and NaCl-containing Petri dishes (**Fig. 2A, 2B**).

The scanned image is shown in **Fig. 3** and the eight morphological indicators measured appear in **Fig. 4** and **5**. **Table 1** shows extreme values observed.

The statistical package-generated discriminant functions are shown in **Fig. 6**. Two variables were excluded: convex area and solidity. Requirements of this kind of analysis were met. Groups of the dependent variable were mutually excluded: cultivars had been previously classified as tolerant or susceptible (**Fig. 2B, 3**). Therefore, the dependent variable was not metrical but categorical. Independent variables were all metrical. The number of cases (100) was higher than twice the number of variables (8).

Repeated observations of each cultivar of the original matrix were evaluated in these discriminant functions to test the accuracy of the functions obtained (**Table 2**). Functions classified correctly 100% (10 bean cultivars) of tolerant or susceptible originally grouped cultivars (**Fig. 2B**).

The Fisher's linear discriminant functions shown in this paper (**Table 3**) are important tools for those bean-breeding programs focused on the production of salinity tolerant plants. Seeds from new bean genotypes can be scanned as described here. Data are evaluated in both discriminant functions. If the resulting value of the tolerant-discriminant function is statistically higher than that of the susceptible discriminant function, then the new bean genotype can be regarded as putatively tolerant. Although the new genotype tolerance still requires additional confirmation under field environment, the results described here allow some research cost reductions because there is no inclusion of a large number of susceptible cultivars.

In addition to the practical use of the assay described

Table 1 Maximal and minimal values (pixels) recorded in the experiment and used for data standardization according to Kantardzic (2003).

	Area	Equivalent diameter	Major axis length	Minor axis length	Eccentricity	Convex area	Solidity	Extent
Maximal value recorded	76876	312860293	433776876	231143471	0.880834	79445	0.99136	0.82889
Minimal value recorded	20705	162365097	197904497	126049564	0.675773	21034	0.95358	0.60373

Table 2 Classification of cultivars as susceptible or tolerant made by Fisher's linear discriminant functions.

Bean cultivar	Result of discriminant function for susceptible cultivars (average ± SE)		Result of discriminant function for tolerant cultivars (average ± SE)	Classification according to the Fisher's linear discriminant functions*
35	74.27 ± 0.80	<	92.28 ± 2.00	Correct classification as tolerant
28	81.65 ± 4.38	<	105.76 ± 3.72	Correct classification as tolerant
3	55.57 ± 1.80	<	76.82 ± 1.51	Correct classification as tolerant
69	58.49 ± 2.97	<	79.59 ± 1.67	Correct classification as tolerant
64	59.55 ± 3.33	<	82.20 ± 2.06	Correct classification as tolerant
83	63.27 ± 2.26	>	41.50 ± 3.13	Correct classification as susceptible
24	65.95 ± 1.25	>	47.41 ± 2.77	Correct classification as susceptible
84	56.10 ± 2.37	>	31.47 ± 3.78	Correct classification as susceptible
87	75.19 ± 1.17	>	57.30 ± 2.88	Correct classification as susceptible
82	73.89 ± 2.22	>	49.61 ± 4.52	Correct classification as susceptible

* These classifications agree with **Fig. 2B**

Table 3 Fisher's linear discriminant functions to differentiate tolerant and susceptible bean cultivars.

Function for susceptible cultivars	S:	- 186.99	*	a	- 1582.05	*	b	+ 779.42	*	c	+ 992.80	*	d	+ 145.40	*	e	+ 14.36	*	f	- 68.27
Function for tolerant cultivars	T:	- 428.15	*	a	- 1092.89	*	b	+ 729.82	*	c	+ 869.63	*	d	+ 111.29	*	e	+ 15.13	*	f	- 88.72

Legend: a: Area; b: Equivalent diameter; c: Major axis length; d: Minor axis length; e: Eccentricity; f: Extent

here, we reached two unexpected conclusions. Firstly, bean seeds were not so NaCl-sensitive during early stages of germination. Most experiments to screen tolerance to sodium chloride used < 150 mmol/L, e.g. Meloni (2003) with cotton and Atia *et al.* (2006) with *Crithmum maritimum* seeds. In contrast, we observed radical emergence with 360 mmol/L.

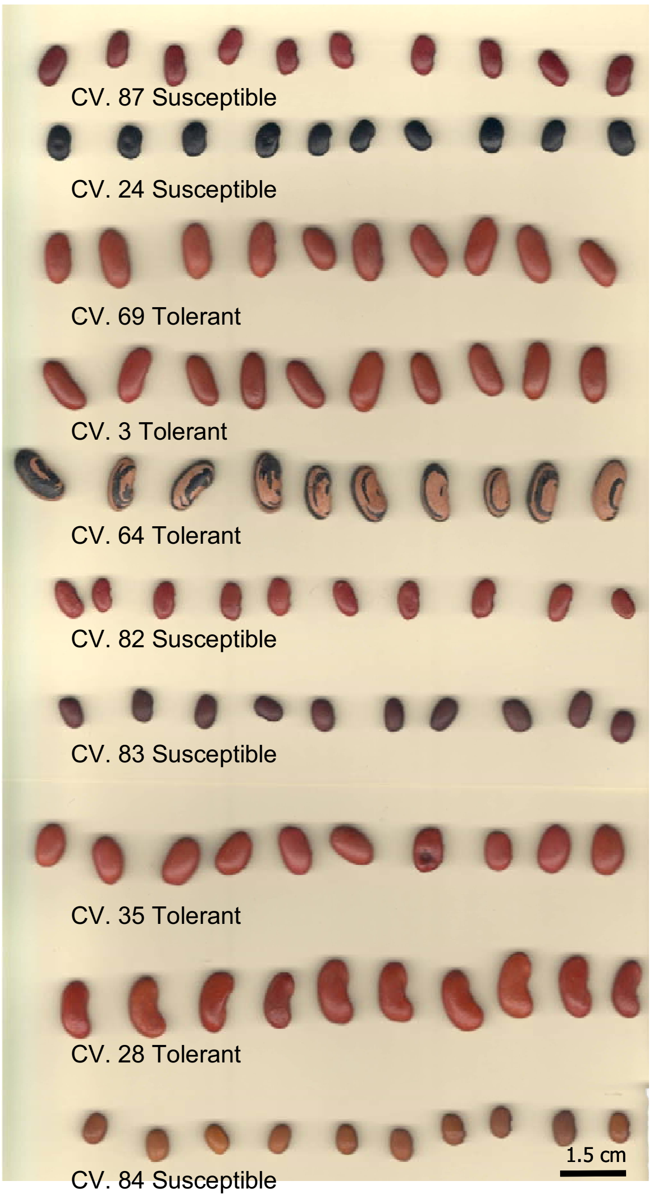

Fig. 3 Bean seeds.

Our second and more important conclusion was: the larger the bean seed size, the lower the sensitivity to sodium chloride. Larger seeds contain more hydrophilic compounds to make imbibition possible (Winn 1985; Vleeshouwers *et al.* 1995; Vaughton and Ramsey 1998; Leishman *et al.* 2000; Cordazzo 2002; Parciak 2002; Dyer 2004; Van Mölken *et al.* 2005). At present, bean cultivars with potential salinity tolerance are being evaluated in different marginal farming communities of Ciego de Ávila (Cuba).

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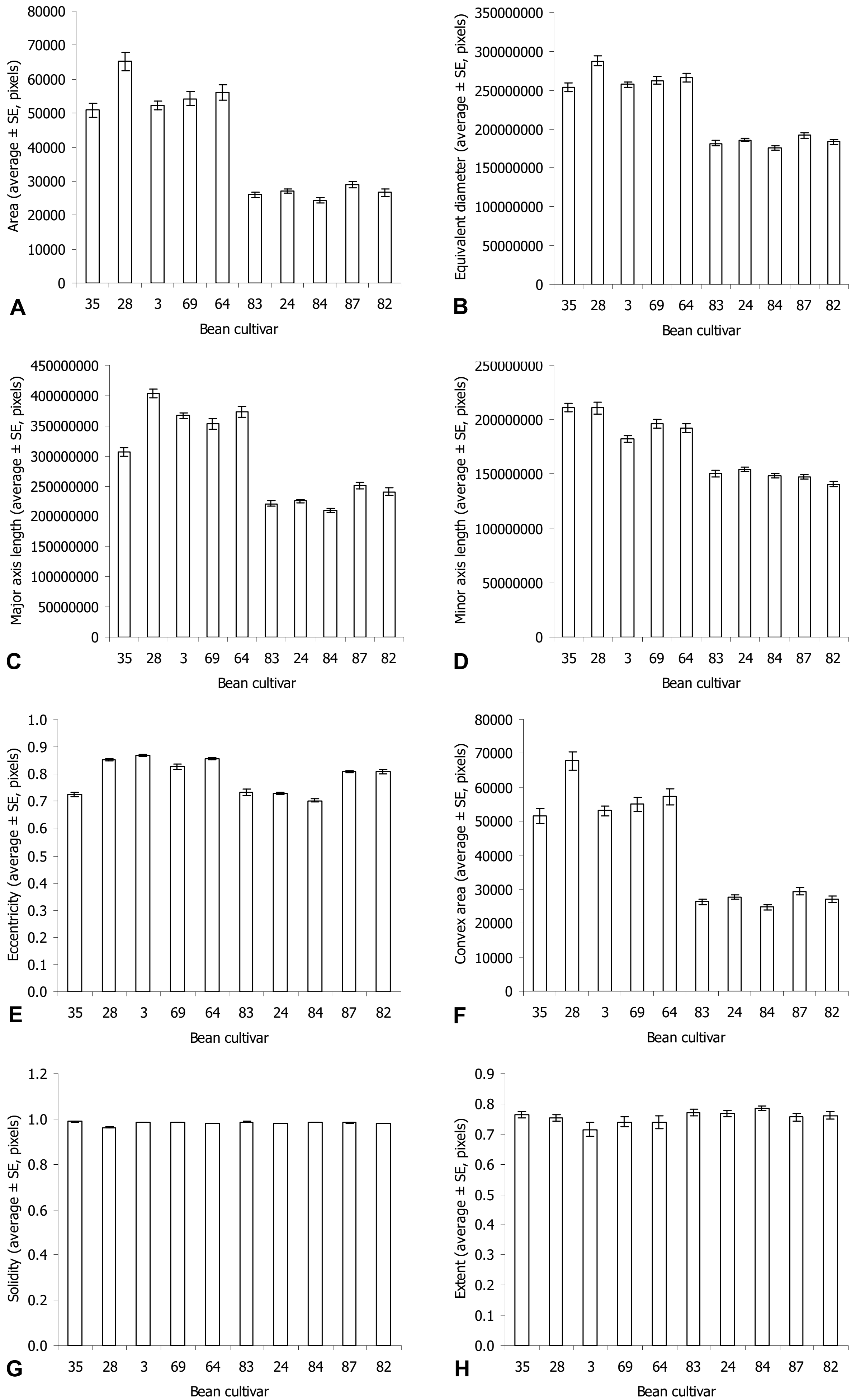

Fig. 4 Morphological indicators of bean seeds.

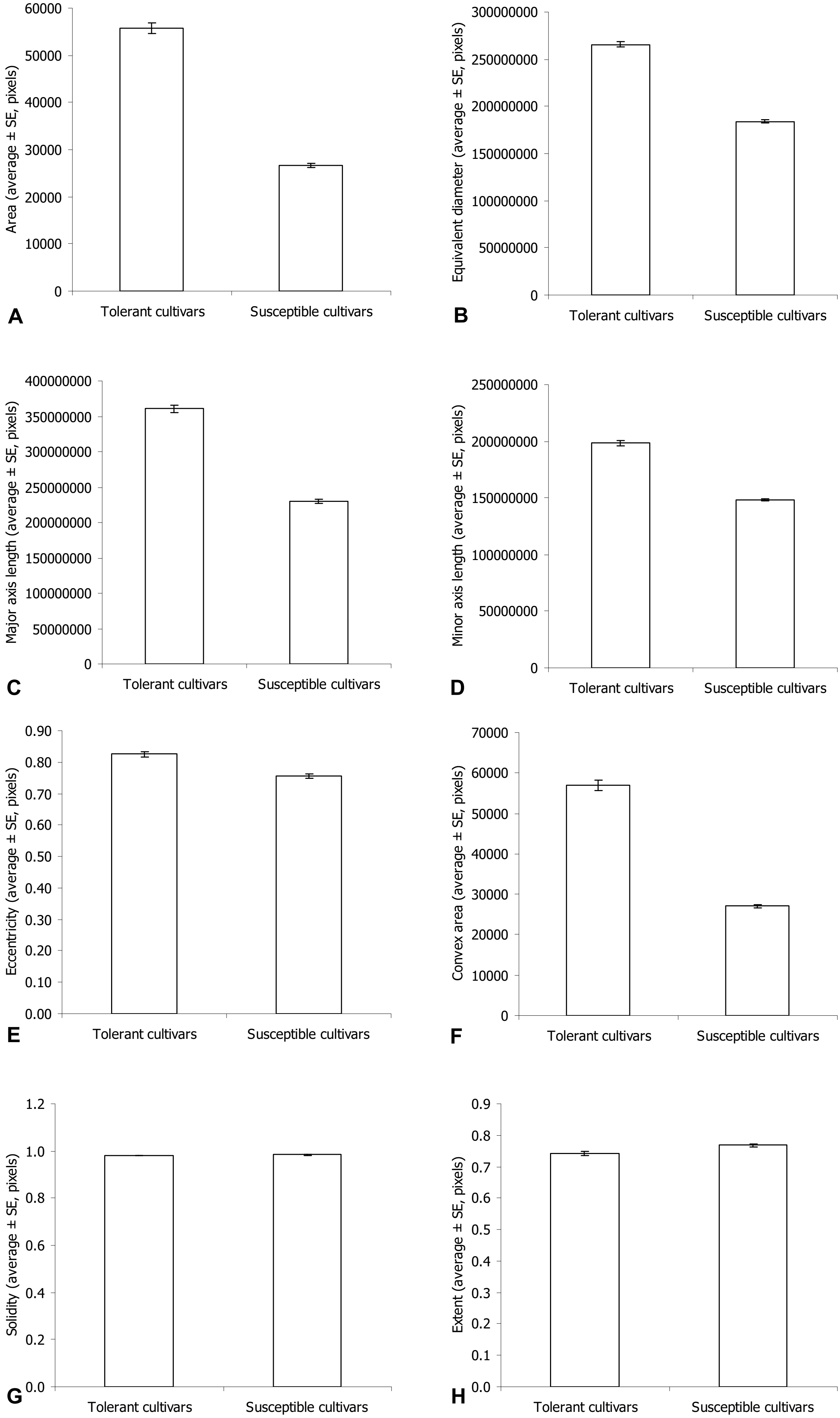

Fig. 5 Summarized morphological indicators of bean seeds.

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