



## Variability of seed quality traits in wild and semi-wild accessions of castor collected in Spain



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### ABSTRACT

The Mediterranean Basin is an important center of diversity for castor (*Ricinus communis* L.). We conducted seed collections from 121 wild and semi-wild (feral) populations growing in southern and eastern provinces of Spain. The objective of this research was to evaluate seed weight, oil content, fatty acid profile, and tocopherol and phytosterol contents and profiles in this germplasm collection at different environments. The seeds collected in the source locations as well as those produced in seed increases conducted in Córdoba (southern Spain) and Murcia (southeastern Spain) were analyzed. Analysis of variance considering three locations (source location, Córdoba, Murcia) and the genotypes was conducted. The contribution of genotype to total variation was particularly high for hundred-seed weight (89.8% of total sums of squares) and concentrations of  $\Delta^5$ -avenasterol (85.4%) and  $\beta$ -sitosterol (79.2%). Large variability was identified for most of the traits. The ranges of variation, averaged from data of at least two environments, were 11.6–59.1 g for hundred-seed weight, 44.6–54.8% for oil content, 99.6–282.0 mg kg<sup>-1</sup> seed kernel for tocopherol content, 27.4–50.5% for the concentration of  $\delta$ -tocopherol, 1090–2865 mg kg<sup>-1</sup> seed for phytosterol content, and 15.1–54.1% for the concentration of  $\Delta^5$ -avenasterol. Little variation was found for fatty acid profile. One accession (ALM-2011-37) showed exceptionally high phytosterol content and three accessions from the same locality (CAD-2010-26 through 28) had high levels of  $\Delta^5$ -avenasterol. The results emphasized the potential of wild and semi-wild germplasm for improving seed and oil quality in castor.

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### 1. Introduction

Castor (*Ricinus communis* L.) is an oilseed crop of the spurge family (Euphorbiaceae). The species is most likely indigenous of East Africa, with the main center of diversity in Ethiopia and additional centers in Northwest Asia, the Mediterranean and Southwest Asia, India, and China (Vavilov, 1951; Moshkin, 1986). Nowadays castor is worldwide distributed in warm regions (Weiss, 2000).

Castor is a minor crop, with around 1.5 million ha cultivated worldwide, mainly in India, China, Brazil, and Mozambique (Fernández-Martínez and Velasco, 2012). The crop is primarily cultivated because of the high oil content of the seeds, which ranges from 40 to 60% (Wang et al., 2010; Fernández-Martínez and Velasco, 2012). The oil is largely made up of ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), a monounsaturated hydroxy fatty acid

that makes the oil unsuitable for edible uses but of great value for industrial uses such as the manufacture of paints, coatings, inks, and lubricants as well as a starting material for many industrial chemical products (Ogunniyi, 2006). Ricinoleic acid typically ranges between 78 and 90% of the total seed oil fatty acids in conventional germplasm, with the other major fatty acids oleic and linoleic acid showing maximum values below 10% (Wang et al., 2011; Fernández-Martínez and Velasco, 2012). Mutant high oleic acid material produces a completely different oil type, with around 12% ricinoleic acid and 81% oleic acid (Rojas-Barros et al., 2004).

Tocopherols are lipid-soluble antioxidants with *in vivo* and *in vitro* free-radical scavenging capacity. *In vivo* antioxidant activity of tocopherols is known as vitamin E activity and results in an effective protection of cellular tissues from oxidative, proliferative, and inflammatory damage (Galli and Azzi, 2010). *In vitro*, tocopherols protect unsaturated fatty acids from oxidation (Shahidi and Zhong, 2010). There are four natural tocopherols, named as  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol, which differ in their chemical structure and antioxidant properties; whereas,  $\alpha$ -tocopherol has maximum vitamin E

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activity,  $\gamma$ - and  $\delta$ -tocopherol are more powerful *in vitro* antioxidants, particularly at high temperatures (Marmesat et al., 2008). Castor seeds contain a high tocopherol content mainly made up of  $\gamma$ - and  $\delta$ -tocopherol (Velasco et al., 2005), though no studies on variability for this trait in castor germplasm have been conducted. There is also no information on variability for phytosterols in castor germplasm. Phytosterols are compounds with similar chemical structure and biological function as cholesterol that play an important nutritional role by reducing cholesterol absorption and consequently blood cholesterol levels in humans (Plat and Mensink, 2005). The cholesterol-lowering properties of phytosterols have stimulated their use as ingredients for a wide spectrum of fortified foods (Zawistowski, 2010). Despite castor oil is an industrial oil, the recovery of phytosterols from the oil and oil distillates would contribute to the value of co-products of the castor oil industry.

Castor is present in Spain mainly in coastal areas of the South and the East. There are two main groups of populations: wild populations, generally with small plants, dehiscent capsules, and small seeds, and semi-wild (feral) populations, with tall plants, usually reddish, indehiscent capsules, and large seeds. The latter group is mainly derived from ornamental plants. Additionally, intermediate and recombinant types, probably derived from hybridization between the two main groups, are also present. These wild and semi-wild castor populations constitute very valuable sources of germplasm of potential use in breeding programs (Auld et al., 2009). From 2010 to 2012 we collected seeds from 121 wild and semi-wild populations of castor in southern and eastern Spain. Preliminary data on plant and seed characteristics from collected accessions were already anticipated (Pascual-Villalobos et al., 2014). The objective of this research was to evaluate the germplasm collection at different environments for seed weight, oil content, fatty acid profile, and tocopherol and phytosterol contents and profiles.

## 2. Materials and methods

### 2.1. Plant material

The collection consisted of 121 accessions collected in 11 provinces from southern and eastern Spain, mainly close to coastal areas (Fig. 1), from 2010 to 2012. Seeds of the accessions and their passport information are available at the seed bank of the Plant Genetic Resources unit of the Spanish National Institute for Agricultural Research and Experimentation (INIA; <http://wwwsp.inia.es/en-us/Investigacion/centros/crf/Paginas/CRF.aspx>). The complete list of accessions used in this research is given in Supplementary Table 1. Roughly 500 seeds were collected per accession. However in some cases, particularly for dehiscent accessions, the number of seeds was considerably lower. Accessions were grown in the experimental farms of IAS-CSIC in Córdoba and IMIDA in Murcia for seed increase and characterization from 2011 to 2013. The study was based on the analysis of the seeds collected in the source locations as well as those obtained in seed increases conducted in Córdoba and Murcia. Because of the high number of accessions and the difficulty of managing the wild and feral accessions, only part of the collection was multiplied every year, *i.e.*, not all the accessions were grown under the same environment at each location. Also, the accessions grown each year were not necessarily the same at both locations. The plants were grown in single 8-m long rows with 1-m separation between rows and plant separation around 33 cm, *i.e.*, around 24 plants per accession. The plants were irrigated periodically until maturity. Before flowering, several racemes per plant were bagged with Kraft paper bags as described by Fernández-Martínez and Velasco (2012) to avoid cross-pollination between accessions. Seeds were collected at maturity and bulked per accession.



Fig. 1. Number of accessions of wild and semi-wild castor accessions collected by province in Spain. Province codes: H, Huelva; SE, Sevilla; CO, Córdoba; CA, Cádiz; MA, Málaga; GR, Granada; AL, Almería; MU, Murcia; A, Alicante; V, Valencia.

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