

# Cowpea: a legume crop for a challenging environment

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## Abstract

**Cowpea is a grain legume native from Africa and is a primary source of protein for millions of people in sub-Saharan Africa and other parts of the developing world. The main important characteristics of this crop include a good protein quality with a high nutritional value, its nitrogen-fixing ability, and an ability to be more drought- and heat-tolerant than most of its legume relatives. In a research perspective, studies of cowpea are relatively scarce, despite its relevance to agriculture in the developing world and its resilience to stress. The present review provides an overview of different aspects of cowpea, with a special emphasis on the molecular markers for assessing genetic diversity, as well as on biochemical and transcriptomic data with respect to evaluating cowpea drought stress tolerance. The integration of both datasets will be useful for the improvement of cowpea because research on drought stress tolerance is of major interest for this crop in a challenging environment.**

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**Keywords:** cowpea; genetic diversity; morphological traits; molecular markers; drought stress; gene expression

## INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a member of Leguminosae family native from Africa and is currently one of the most important grain legumes growing in tropical and subtropical regions.<sup>1–3</sup> This legume has been used in the human diet, as well as in forage for animal feeding. For human consumption, the most important product is the dry grain that can be consumed boiled, fried (as akara) or steamed (as moi moi),<sup>4</sup> according to different preparations, in salads, snacks and cakes, amongst others. Also, young leaves, fresh pods and fresh seeds have been consumed in some world regions.<sup>4,5</sup> Green organs could be used as a vegetable and are often served boiled, as well as being consumed fried or fresh.<sup>5</sup> One of the most important characteristics of cowpea is the high nutritive content value of all plant parts.<sup>3,4,6</sup> The dry grain is rich in proteins (23–32%), as well as essential amino acids such as lysine (427 mg g<sup>-1</sup> N) and tryptophan (68 mg g<sup>-1</sup> N), although it is low in sulphur-containing amino acids.<sup>7,8</sup> Accordingly, cowpea and cereals complement each other in terms of amino acids and, consequently, a diet combining both provides a balanced protein intake. The presence of both minerals (iron and zinc) and vitamins (folic acid and vitamin B) has also been reported to be important in preventing birth defects during pregnancy.<sup>3,9,10</sup> Dry grain is also high in fibre and low in fat.<sup>8</sup> Taking into account these advantages, an increase in cowpea production and consumption in the European Union is highly desirable. Currently, the European Union imports almost all of the cowpea consumed from African countries, more specifically from Niger and Nigeria. During the period 2009–2013, the world cowpea planting area was 5 million hectares and the worldwide production was 12 million tonnes. Africa has been responsible for 95.4% of worldwide cowpea production,<sup>11</sup> with the drier savannah and the Sahelian region of West and Central Africa being responsible for producing 72% of the total. Nigeria and Niger are the largest producers, with 3.4 and

1.4 million tonnes, respectively. By contrast, Europe is only responsible for 0.4% of worldwide cowpea production and the European Union has only produced 463 thousand tonnes during the period 2009–2013.<sup>11</sup>

As revealed by the major producing countries, cowpea has the capacity to grow in low fertility soils, which is related to its ability to establish associations with distinct microorganisms, mainly nitrogen-fixing bacteria (e.g. rhizobia) and vesicular-arbuscular mycorrhizal fungi. Cowpea tolerance to low fertility soils<sup>8,12,13</sup> and a wide range of soil pH,<sup>14</sup> as well as the adaptation of cowpea to high temperatures and drought,<sup>15</sup> makes this grain legume crop of interest for facing the predicted environmental changes (e.g. increased temperature, reduction of water availability) associated with climate change. The present review provides an overview of different issues about genomic and transcriptomic studies in cowpea, with an emphasis on studies related to genetic diversity and cowpea drought stress tolerance that could be useful with respect to integration in cowpea breeding programs.

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**Table 1.** Taxonomic classification of cowpea

Species	Subspecies	Variety	Cultivar group
<i>Vigna unguiculata</i>	<i>unguiculata</i>	<i>Spontanea</i>	<i>unguiculata</i> <i>biflora</i> <i>sesquipedalis</i> <i>textilis</i> <i>melanophthalmus</i>
		<i>unguiculata</i>	
		<i>baoulensis</i>	
		<i>burundiensis</i>	
	<i>letozeyi</i>	<i>aduensis</i>	
		<i>pawekiae</i>	
		<i>dekindtiana</i>	
		<i>stenophylla</i>	
		<i>tenuis</i>	
		<i>alba</i>	
		<i>pubescens</i>	

## CLASSIFICATION AND DESCRIPTION

The cowpea cultivated form obtained from the Antilles was first described by Linnaeus as *Dolichos unguiculatus* L., later being classified by Walpers as *Vigna unguiculata* (L.) Walp.<sup>16</sup> This diploid species ( $2n = 2x = 22$ ) belongs to the division Magnoliophyta, class Magnoliopsida, order Fabales, family Leguminosae, tribe Phaseoleae, genus *Vigna*. The genus *Vigna* includes more than 80 species<sup>17</sup> and was subdivided into six sections, namely, *Vigna*, *Comosae*, *Macrodonatae*, *Reticulatae*, *Liebrechtsia* and *Catiang*.<sup>18</sup> *Vigna unguiculata* (L.) Walp. includes annual cowpeas (ssp. *unguiculata*) and ten wild perennial subspecies (Table 1).<sup>19</sup> The subspecies *unguiculata* includes all the domesticated forms (var. *unguiculata*), as well as the wild and weedy forms [var. *spontanea* (Schweinf.) Pasquet].<sup>19,20</sup> The domesticated forms are subdivided into four cultivar-groups essentially based on seed and pod characters.<sup>19,21</sup> These cultivar-groups are *unguiculata* grown as pulse, *biflora* (catjang) used mainly as forage, *sesquipedalis* (asparagus bean) grown as a vegetable, and *textilis* cultivated for the fibres of its long floral peduncles.<sup>19</sup> Pasquet<sup>16</sup> also proposed the insertion of *melanophthalmus* (black-eyed pea) as another cultivar-group.

'Cowpea' is the *V. unguiculata* most popular worldwide name, although local names such as black-eyed beans, black-eyed peas, pink-eyes or southern peas (all used in the USA), 'frijol caupi' (Spanish speaking countries in America), 'lobia' (India), 'caupi' (Brazil), 'caupi' and 'carilla' (Spain), 'niébé' (French speaking countries of Africa) and 'feijão-frade' (Portugal) are used.

Cowpea is described as an herbaceous warm-season annual plant with a great variability in morphology. This crop is autogamous but approximately 5% outcrossing was reported in the cultivated varieties probably as a result of insect activities.<sup>17,22</sup> Its growth habit could be prostrate (trailing), semi-prostrate, semi-erect, erect or climbing, depending not only mostly on genotype, but also on photoperiod and growth conditions, with the pattern of growth being determinate or indeterminate.<sup>8,23</sup> This crop is well adapted to a wide range of soil types from sands to heavy, including low fertility soils.<sup>24</sup> Plants grow in an extensive range of temperatures, with 28 °C the optimal temperature. Early flowering cowpea can produce a crop of dry grain in only 60 days, whereas longer season cowpeas may require more than 150 days to produce mature pods, depending on photoperiod.<sup>8</sup>

According to the International Institute of Tropical Agriculture (IITA) and Bioversity International (ex-International Board for Plant Genetic Resources; IBPGR), the leaves can be classified into four categories: sub-globose, sub-hastate, globose and hastate/lanceolate.<sup>23</sup> Flowers emerge in alternate pairs on racemes at the distal ends of long peduncles, with usually two flowers per inflorescence. Flowers have a short life cycle, opening in the early day and closing at approximately midday, after which they usually wilt and collapse.<sup>25</sup> Corollas can be purple, mauve–pink, yellow or white.<sup>23</sup> Each peduncle commonly develops two or three pods and pods differ in size, shape, colour and texture.<sup>8</sup> They are cylindrical, although they could be straight, slightly curved, curved or coiled and, when they ripe, the colour can vary from yellow to brown or dark purple.<sup>23</sup> The sub-species/cultivar-group *Sesquipedalis* (more common in Asia) have very long green pods (40–100 cm) that are often used as green beans (or snap beans),<sup>8</sup> whereas the other groups have standard pods (10–25 cm). Seeds differ in size and colour, ranging from white, cream, green, buff, red, brown or black and can be kidney, ovoid, crowder, globose or rhomboid and are characteristic by the presence of an eye, as a result of the different pigmentations encircling the hilum.<sup>23</sup>

Environmental conditions, including photoperiod and growing conditions (temperature, rainfall, etc.), can also affect the plant height and morphology.<sup>8,24</sup> Cowpea root system is dense and well-developed<sup>26</sup> and has a beneficial effect on the structure and tilth of the topsoil layer. Most root growth occurs within the topsoil layer but, in drought conditions, a long taproot can grow for reaching the deeper moisture in the soil profile.<sup>27</sup> These characteristics furnish cowpea plants with a high resistance to drought in comparison with other legumes.

## ORIGIN, DOMESTICATION AND DISTRIBUTION

Africa was suggested as the centre of origin of cowpea.<sup>28</sup> This assumption was not contested because wild cowpea plants have been found in tropical Africa and Madagascar,<sup>1</sup> where it was presumably domesticated subsequent to the Neolithic age.<sup>29</sup> Pasquet<sup>30</sup> suggested that the most likely progenitor of domesticated cowpea is *V. unguiculata* ssp. *unguiculata* var. *spontanea*. For determining the precise domestication site and the cowpea diversity centres, several studies have been performed in the last decades, although a conclusive result has been difficult to reach. Several hypotheses have been proposed for cowpea domestication, such as Ethiopia,<sup>1,31,32</sup> West Africa,<sup>33–37</sup> and Eastern and Southern Africa.<sup>38</sup> Coulibaly *et al.*,<sup>19</sup> using amplified fragment length polymorphisms (AFLPs) and morphologic data, concluded that the wild species was originated from Eastern Africa. In this case, domestication should have occurred in Northeastern Africa and the domesticated plant was then probably dispersed to Western Africa. According to Ng and Padulosi,<sup>39</sup> West Africa appears to be the centre of diversity of cultivated forms. A 'diffuse' domestication in the African savanna after the dispersal of cereals was also hypothesized.<sup>1,40</sup> This last hypothesis was presented by Harlan,<sup>41</sup> who considered that the cowpea was domesticated in the African Non-Center. Whatever the place of domestication, cowpea is an ancient legume that was domesticated by African gatherers, cultivators and farmers from its wild forms in Africa dating back to Neolithic times.<sup>2</sup> During the Neolithic period, the cowpea was first introduced into India, which was then considered a secondary centre of cowpea genetic diversity.<sup>42</sup> The spread of cowpea in Asia occurred at the end of Neolithic period (third millennium BC), where the subspecies asparagus bean or yardlong (*V. unguiculata*

*ssp. sesquipedalis*) is still cultivated for long immature pods,<sup>43</sup> as well as in America between the 16th and 17th centuries (AD).<sup>44</sup> Although some reports suggest that cowpea has been cultivated in Europe at least since the 18th century BC and possibly from prehistoric times onward,<sup>19,45</sup> others suggest that it was only introduced in Europe around 300 BC, where it still remains as a minor crop in the southern part.<sup>17</sup> From Europe, more specifically from Portugal and Spain, this legume was exported in the 17th century to the New World.<sup>17,46</sup> Another important result was obtained by Fang *et al.*<sup>46</sup> who provided evidence for the common origin of cowpea germplasm from Asia and North America different from the West Africa. However, such studies have mostly used breeding lines and, consequently, the introgression of extra regional germplasm could have occurred. Huynh *et al.*,<sup>47</sup> analysing a worldwide collection of cowpea landraces and African ancestral wild cowpeas by using more than 1200 single nucleotide polymorphism (SNP) markers, confirmed that accessions from Asia and Europe were more related to those from western Africa, whereas accessions from Americas appeared to be more closely related to those from Eastern Africa.

## EVALUATION OF GENETIC DIVERSITY

Cowpea has been referred as a worldwide crop with more prevalence in tropical areas, displaying a high phenotypic/morphological variability.<sup>8</sup> Genetic diversity assessment is then useful for the preservation and utilization of germplasm resources, as well as for the improvement of varieties/cultivars.<sup>3</sup> Genetic diversity can be evaluated using morphological traits, biochemical and molecular markers. Each of these markers has different applications in several areas, such as plant breeding, phylogenetic studies, gene mapping, genetic engineering, micro-propagation and genetic resources characterization, and can be used individually or combined.

Several studies have been referring the characterization of cowpea by morphological and quantitative traits.<sup>16,48–52</sup> This characterization is followed by using a set of descriptors: (i) parameters related to plant morphology, such as growth habit, leaf type, flower colour, seed shape and colour and (ii) parameters related to plant production, namely the number of pods and seeds per plant and seed weight. Morphological characterization does not require any complex equipment or experiments, being simple and inexpensive to score. These reasons explain the constant use of morphological traits as a first step for evaluating genetic relationships. The main disadvantage is that the observed characteristics do not exclusively reflect the genotype but, instead, reflect the interaction between genotype and environment.<sup>53</sup>

The first biochemical markers to be used for genetic diversity analysis were the isozyme markers in the 1960s.<sup>54</sup> These enzymes differ in amino acid sequence and are encoded by different genetic *loci* (isozymes) or by different alleles at the same *locus* (allozymes), yet catalyse the same reaction.<sup>55</sup> Until the end of 1980s, isozymes were the main marker used to analyse the genetic variability and taxonomy in plants, helping to define the phylogenetic relationships and population genetics. Over the years, several studies were developed in cowpea that made use of this biochemical marker. Panella and Gepts<sup>56</sup> and Vaillancourt *et al.*<sup>57</sup> characterized wild and cultivated accessions of cowpea by using 10 and 26 isoenzyme *loci*, respectively, and concluded that the genetic diversity in the evaluated collections was low. Besides isozyme markers, seed storage protein profiling is another method used to reveal genetic variation between cowpea cultivars.<sup>58–62</sup> Often, in these studies, the

obtained results were not very conclusive as a result of a lack of domesticated cowpea and progenitor representative samples.

In comparison with morphological and biochemical markers, DNA molecular markers have a set of characteristics that make them ideal to several studies, such as their highly polymorphic nature and frequent occurrence in the genome, allowing a direct comparison of genetic material in an environmental independent way.<sup>54,63</sup> DNA-based molecular markers have been extensively used in cowpea genetic diversity research, variety identification, phylogenetic analysis, gene mapping and resource classifications (Table 2). The first study using AFLP markers in cowpea was performed by Coulibaly *et al.*,<sup>19</sup> in which the genetic relationship among a total of 117 cowpea accessions [including 47 domesticated cowpeas (*ssp. unguiculata*) and 52 wild and weed annuals (*ssp. unguiculata* var. *spontanea*)] was investigated. It was shown that the wild cowpeas were more diverse than domesticated ones, and an Eastern African origin for the wild taxon was also suggested. This result was corroborated by Ba *et al.*<sup>2</sup> using random amplified polymorphic DNA (RAPD) markers, and by Ogunkanmi *et al.*<sup>64</sup> with single sequence repeat (SSR) or microsatellites markers. The variation within and among cowpea populations from different agro-ecological regions and germplasm accessions has been also evaluated using AFLP<sup>46</sup> and RAPD markers.<sup>65–68</sup> In addition, RAPD markers were used to eliminate the putative duplicates of Senegal cowpea accessions in a germplasm bank and identify elite varieties.<sup>69</sup> Currently, SSR is the most frequently used molecular marker in cowpea genetic diversity analyses, namely in cowpea landrace accessions from China, Africa and other Asian countries,<sup>70</sup> Korea,<sup>71</sup> Ghana,<sup>72</sup> Southwestern Nigeria,<sup>73</sup> and Senegal,<sup>74</sup> where a high genetic diversity was observed. To evaluate the genetic diversity of asparagus bean (*V. unguiculata* *ssp. sesquipedalis*) cultivars from different Chinese geographical origins, SSR markers derived from *V. unguiculata* *ssp. unguiculata* sequences were used, confirming the transferability of SSR markers between these two subspecies.<sup>75</sup> In all of these studies, SSR markers also showed sufficient genetic variance that could be useful for improvement strategies in cowpea. SNP markers have gained an increasing importance because of their bi-allelic nature, higher frequency in the genome than SSRs and other markers, and their easily automated genotyping.<sup>76</sup> In a study of the characterization of 113 cowpea accessions, comprising 108 from Ghana and five from abroad, 458 SNPs (out of 477) showed high polymorphism.<sup>77</sup> These results suggest an unexpected high level of heterozygosity. The chip-based SNP detection technology is being widely used in plant genetic applications.<sup>78–80</sup> In cowpea, Illumina chip-based SNP detection platforms (GoldenGate and more recently iSelect; Illumina, San Diego, CA, USA) have been developed and are proving very useful for molecular characterization,<sup>77,81</sup> genetic diversity analysis<sup>47,82</sup> and genetic mapping.<sup>43,83–85</sup> Researchers at the University of California, Riverside, in partnership with institutions from several African countries, have designed a 60 000-assay iSelect BeadArray for cowpea that successfully assayed 51 128 SNPs.<sup>86</sup>

The combined use of different molecular markers could better assist the evaluation of genetic diversity. Diouf and Hilu<sup>87</sup> used a combination of RAPD and SSR markers to assess genetic variability of local cowpea varieties and breeding lines from Senegal and identified 12 polymorphisms as a result of the broad genome coverage used. Combinations of AFLP and SAMPL (selectively amplified microsatellite polymorphic locus) markers,<sup>88</sup> as well as AFLP and SSR markers,<sup>89</sup> were used to determine the genetic variation within and among closely related *V. unguiculata* accessions, whereas the combined use of RAPD and ISSR markers allowed the

**Table 2.** DNA-based molecular markers that have been used for specific cowpea studies

Molecular marker	Sub-species	Objective	Reference
AFLP	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to cowpea parasitism resistance	Ouédraogo et al. (2001) <sup>153</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Phenetic organization and genetic diversity	Coulibaly et al. (2002) <sup>19</sup>
	<i>V. unguiculata</i> ssp. <i>spontanea</i>	Genetic diversity	Fang et al. (2007) <sup>46</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to cowpea golden mosaic virus	Rodrigues et al. (2012) <sup>154</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to orthologous seed weight genes	Fatokun et al. (1992) <sup>155</sup>
RFLP	<i>V. unguiculata</i> ssp. <i>unguiculata</i> <i>Vigna radiata</i>	Markers linked to aphid resistance gene	Myers et al. (1996) <sup>156</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Diversity of indigenous bradyrhizobia	Krasova-Wade et al. (2003) <sup>157</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to genotypic and phenotypic responses to seedling-stage drought	Muchero et al. (2008) <sup>100</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Fall et al. (2003) <sup>69</sup>
RAPD	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic relatedness and gene flow	Nkongolo (2003) <sup>158</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Ba et al. (2004) <sup>2</sup>
	<i>V. unguiculata</i> ssp. <i>Spontanea</i>	Genetic diversity	Zannou et al. (2008) <sup>65</sup>
	<i>Vigna unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity and markers linked to cowpea resistance to pests weevil pests	Abdel-Sabour et al. (2010) <sup>159</sup>
	<i>Phaseolus vulgaris</i>	Genetic diversity	Malviya et al. (2012) <sup>66</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Prasanthi et al. (2012) <sup>67</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Patil et al. (2013) <sup>68</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity and relationships	Li et al. (2001) <sup>105</sup>
	<i>V. unguiculata</i> ssp. <i>dekindtiana</i> var. <i>pubescens</i>	Genetic diversity	Xu et al. (2007) <sup>70</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Ogunkanmi et al. (2008) <sup>64</sup>
SSR	<i>V. unguiculata</i> ssp. <i>ovata</i>	Genetic diversity	Lee et al. (2009) <sup>71</sup>
	<i>V. unguiculata</i> ssp. <i>kgalagadensis</i>	Genetic diversity	Asare et al. (2010) <sup>72</sup>
	<i>V. unguiculata</i> ssp. <i>rhomboidea</i>	Genetic diversity and SSR transferability between <i>Vigna</i> species	Gupta and Gopalakrishna (2010) <sup>160</sup>
	<i>V. unguiculata</i> ssp. <i>Pubescens</i>		
	<i>V. unguiculata</i> ssp. <i>mensensis</i>		
	<i>V. unguiculata</i> ssp. <i>grandiflora</i>		
	<i>V. unguiculata</i> ssp. <i>Congolensis</i>		
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Lee et al. (2009) <sup>71</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Asare et al. (2010) <sup>72</sup>
	<i>V. vexillata</i>	Genetic diversity and SSR transferability between <i>Vigna</i> species	Gupta and Gopalakrishna (2010) <sup>160</sup>
	<i>V. umbellata</i>		
	<i>V. glabrescens</i>		
	<i>V. aconitifolia</i>		
	<i>V. trilobata</i>		
	<i>V. angularis</i>		
	<i>V. radiata</i>		
	<i>V. radiata</i>		
	<i>V. radiata</i> var. <i>setulosa</i>		
	<i>V. radiata</i> var. <i>sublobata</i>		
	<i>V. mungo</i>		
	<i>V. mungo</i> var. <i>Silvestres</i>		
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity of cowpea cultivars resistant to <i>Striga gesnerioides</i>	Sawadogo et al. (2010) <sup>101</sup>
	<i>V. unguiculata</i> ssp. <i>sesquipedalis</i>	Genetic diversity and SSR transferability between sub-species	Xu et al. (2010) <sup>75</sup>
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic distance and diversity	Adewale et al. (2011) <sup>48</sup>	
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic map and identification of QTLs	Andargie et al. (2011) <sup>96</sup>	
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to Yellow Mosaic Virus Resistance genes	Gioi et al. (2012) <sup>161</sup>	
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	SSR transferability to other <i>Vigna</i> species	Bansal et al. (2012) <sup>162</sup>	
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Badiane et al. (2012) <sup>74</sup>	
<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Adetiloye et al. (2013) <sup>73</sup>	



**Table 2.** DNA-based molecular markers that have been used for specific cowpea studies

Molecular marker	Sub-species	Objective	Reference
AFLP	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to cowpea parasitism resistance	Ouédraogo <i>et al.</i> (2001) <sup>153</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Ali <i>et al.</i> (2015) <sup>163</sup>
SNP	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Consensus genetic linkage maps	Muchero <i>et al.</i> (2009) <sup>83</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Linkage mapping and synteny to other legumes	Lucas <i>et al.</i> (2011) <sup>84</sup>
	<i>Glycine max</i>		
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to resistance to foliar thrips	Lucas <i>et al.</i> (2012) <sup>104</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Gene pool structure	Huynh <i>et al.</i> (2013) <sup>47</sup>
	<i>V. unguiculata</i> ssp. <i>dekindtiana</i>	Phylogenetic relationships	
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Markers linked to seed size	Egbadzor <i>et al.</i> (2013) <sup>99</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity	Egbadzor <i>et al.</i> (2014) <sup>52</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic mapping and synteny of aphid resistance	Huynh <i>et al.</i> (2015) <sup>164</sup>
	<i>V. unguiculata</i> ssp. <i>unguiculata</i>	Genetic diversity and population structure	Xiong <i>et al.</i> (2016) <sup>82</sup>
	<i>V. unguiculata</i> ssp. <i>spontanea</i>	Consensus genetic map	Muñoz-Amatriain <i>et al.</i> (2016) <sup>85</sup>
	<i>V. unguiculata</i> ssp. <i>spontanea</i>		
	<i>V. unguiculata</i> ssp. <i>sesquipedalis</i>	Pod length QTLs	Xu <i>et al.</i> (2016) <sup>165</sup>

evaluation of genetic variations of seven *Vigna* species.<sup>90</sup> A combination of molecular and classical markers has been considered essential for making the results of genetic diversity more reasonable with respect to genetic cowpea breeding and the evaluation of germplasm resources.<sup>3</sup> The combined use of molecular markers (SSR and ISSR) and classical markers (morphological traits) was described to estimate the genetic diversity and relatedness of 23 asparagus bean (*V. unguiculata* ssp. *sesquipedalis*) accessions and seven accessions of a hybrid between cowpea (*V. unguiculata* ssp. *unguiculata*) and dwarf asparagus bean in Thailand.<sup>91</sup> Morphological characters were diverse among most accessions, although their exclusive use did not allowed a distinction between accessions. Indeed, ISSR markers showed higher efficiency for estimating the levels of genetic diversity and relationships among the two subspecies than SSR markers.<sup>91</sup> The combined use of morphological traits, RAPD and ISSR markers was also employed for discriminating landraces of cowpea scattered from all Algeria regions,<sup>92</sup> as well as for evaluating the genetic variability and relationships between two cowpea cultivars and nine elite genotypes.<sup>93</sup> Both studies showed that ISSR markers were better linked to morphological variation than RAPD markers.

### Genetic mapping and marker-assisted selection

Currently, the construction of the cowpea genetic map is mainly based on the use of efficient molecular markers, such as SSR and SNP, which show sufficient genetic variability.<sup>43,83,84,94–97</sup> A consensus genetic linkage map using expressed sequence tag-derived SNPs led to the integration of 928 markers into a cowpea genetic map spanning 680 cM with 11 linkage groups (0.73 cM of average marker distance).<sup>98</sup> A significant macrosynteny with *Glycine max* and *Medicago truncatula* genomes was reported, as well as some microsynteny with *Arabidopsis thaliana* genome. The first genetic map of asparagus bean based on SNP and SSR markers was reported by Xu *et al.*<sup>43</sup> This map consisted of 375 *loci* mapped on 11 linkage groups, with 191 *loci* detected by SNP markers and 184 *loci* by SSR markers. The development of a high-density genetic map offers a powerful tool for analysing the inheritance of target genes, as well as monitoring specific genes or genomic regions transmitted from parents to progeny.<sup>3</sup> Using the recently developed Illumina iSelect genotyping assay for

cowpea, Muñoz-Amatriain *et al.*<sup>85</sup> genotyped five biparental recombinant inbred lines (RIL) populations and developed a consensus genetic map containing over 37 000 SNPs mapped to approximately 3200 bins in 800 cM. These results are being used to genetically anchor an initial whole-genome shotgun assembly of the cowpea accession IT97K-499-35. To this assembly, sequences from approximately 4000 minimal tiling path bacterial artificial chromosomes (BAC) are being incorporated with the aim of increasing the number of anchored scaffolds and helping resolve the order within recombination bins.

The biotechnology based on such genetic maps and the use of DNA markers brings great hope to cowpea breeding because specific molecular markers could be used to select target traits with marker assisted selection (MAS).<sup>17</sup> The association of 18 SNPs with seed size in cowpea varieties from Ghana suggested that these molecular markers could be useful for marker assisted breeding of larger seeded cowpea plants.<sup>99</sup> Performing a RFLP analysis of 29 polymorphic markers among 14 drought-tolerant genotypes, it was possible to find a correlation between seven RFLP markers and different drought-related cowpea phenotypes.<sup>100</sup> The additional use of other high-density DNA markers in the genome could speed up the selection process in breeding programs even more. For breeding to resistance to the parasitic weed *Striga gesnerioides*, SSR<sup>101</sup> and AFLP<sup>102,103</sup> markers have been used. Similarly, SNPs have been used to identify markers associated to cowpea resistance to foliar thrip.<sup>104</sup> The asparagus bean rust disease, caused by the fungus *Uromyces vignae*, was also associated with a specific AFLP marker that can now be effectively used for MAS.<sup>105</sup> Sequencing and analysis of the gene-rich hypomethylated portion of the cowpea genome was performed by Timko *et al.*<sup>106</sup> More than 250 000 gene-space sequences reads were generated, thus providing a source of functional markers for detailed comparative studies of cowpea with other plant species and positional cloning of key genes of agronomic interest.

### TOLERANCE TO DROUGHT STRESS

Drought is one the most severe environmental stresses with major impact on plant development and productivity thus causing serious agricultural yield losses.<sup>107,108</sup> Drought tolerance is

a complex trait defined as the ability of plants to live, grow and reasonably produce with limited soil water supply or under periodic water deficiencies.<sup>109</sup> Mitra<sup>110</sup> grouped the plant mechanisms used to cope with drought stress into three groups: drought escape, drought avoidance and drought tolerance. Crop plants could use more than a single mechanism to cope with drought stress. One of the most important food legumes in tropical and sub-tropical regions, where drought is a major constraint for production as a result of low and erratic rainfall, is cowpea. Indeed, some studies noted cowpea to be one of the most tolerant crops to drought as a result of its capacity to grow in areas with no irrigation facilities and irregular rainfall.<sup>24,111–113</sup> This tolerance has been attributed to the three drought tolerance mechanisms,<sup>112</sup> although several drought avoidance mechanisms were extensively described, including deep rooting, strong stomatal sensitivity, reduced growth rate, leaf area reduction, delayed leaf senescence, hastened or delayed reproductive cycle, osmotic adjustment and sensitive moisture remobilization to the upper leaves and growing tips.<sup>109,113</sup> Because cowpea has the ability to tolerate severe drought conditions and displays a relatively small nuclear genome size (estimated at approximately 620 Mb), this legume has been considered as an ideal model for studying the molecular mechanisms of drought tolerance in crops.<sup>112</sup>

### Morphological, biochemical and physiological traits for drought

Changes of morphological, biochemical and physiological traits in response to drought stress for several *V. unguiculata* cultivars have been reported.<sup>114–117</sup> The root system or rooting pattern are closely related to drought-tolerance mechanisms in legume crops.<sup>118,119</sup> To evaluate and screen cowpea drought-tolerance, several parameters of the root system have been used, such as root length density, rooting depth and root dry matter.<sup>119</sup> To examine cowpea drought tolerance ability, water potential, relative turgidity, diffusion pressure deficit, chlorophyll stability index measurements or carbon isotope discrimination are typically evaluated.<sup>109,120</sup> However, most of these methods have the disadvantage of being slow, laborious, expensive and influenced by environmental conditions.<sup>109,112</sup> Slabbert *et al.*<sup>114</sup> tested and proposed other methods that screen cowpea for drought tolerance, such as proline accumulation, 2,3,5-triphenyltetrazolium chloride assays, cell membrane stability, relative water content, leaf water potential, leaf area, chlorophyll *a* and *b* contents, chlorophyll fluorescence, carotenoids content, evaluation of anti-oxidative responses through enzyme activities determination [superoxide reductase, glutathione reductase (GR), ascorbate peroxidase (APX)], as well as the early drought screening at the seedling stage (wooden box technique). Altogether, these methods pretend to evaluate the most typical changes that occur in plants after a drought imposition.

Because the complex regulatory processes of drought adaptation involves the control of water flux and cellular osmotic adjustments via the biosynthesis of osmoprotectants,<sup>108</sup> the determination of such compounds has often been used for screening tolerant cowpea genotypes. The osmoprotectants are classified into three major groups: amino acids (e.g. proline), polyol/sugars (e.g. trehalose, fructans, mannitol) and quaternary amines (e.g. glycine betaine).<sup>121–123</sup> However, these compounds do not accumulate in all plant species in sufficient amounts to avoid adverse effects of drought stress.<sup>122,124</sup> Studies in drought stress cowpea and osmoprotectants are still scarce. However, the

application of chitosan in drought stress cowpea plants has been described to allow the maintenance of osmotic balance.<sup>125</sup>

Physiological changes related to photosynthesis and stomatal conductance have also been frequently used in drought evaluation studies. Indeed, one of the processes largely affected by water deficit is photosynthesis as a result of a decline of stomatal conductance that limits the carbon assimilation, as well as biochemical and photochemical adjustments.<sup>126,127</sup> The dynamics of photosynthesis (*A*), stomatal conductance (*g<sub>s</sub>*) and intrinsic water-use efficiency ( $WUE = A/g_s$ ) were evaluated in 14 cowpea genotypes over a period of drought and post-stress.<sup>128</sup> Under water stress conditions, a decrease in photosynthesis and stomatal conductance accompanied by an increase in the intrinsic water-use efficiency was detected in all genotypes, although differences between genotypes were found.<sup>128</sup> When cowpea genotypes differing in drought resistance were subjected to three distinct water stress conditions (unstressed, moderate and severe stressed), an increase in root biomass and a reduction in chlorophyll content were detected with water stress imposition.<sup>115</sup>

One of the main regulators of plant drought tolerance is the abscisic acid (ABA) that, not only regulates many essential processes of plant development, including the inhibition of germination and control of stomatal closure, but also several adaptive responses to a variety of environmental stresses.<sup>129,130</sup> Kulkarni *et al.*,<sup>131</sup> studying the response of six cowpea cultivars to drought stress, suggested that the intrinsic capacity for ABA synthesis could play an important role in regulating stomatal conductance. ABA accumulation is higher in drought-stressed plants than in unstressed plants.<sup>112</sup> In cowpea, some studies have been developed aiming to understand the role of ABA in the drought tolerance.<sup>132,133</sup>

Because membranes are the key targets of degradative processes induced by drought, membrane integrity parameters have also been used for assessing drought stress severity. A decrease in membrane lipid content was reported under water stress,<sup>134</sup> which appears to be correlated to the inhibition of lipid biosynthesis and stimulation of lipolytic and peroxidative activities.<sup>135,136</sup> The degradation of membrane lipids and the enzymatic antioxidant activity appears to be a useful method for evaluating the level of plant drought stress. However, data are still scarce in cowpea.<sup>111,114,136–138</sup>

Agbidoco *et al.*<sup>112</sup> suggested that the most suitable parameters for screening a large number of cowpea lines for drought tolerance are the measurements of chlorophyll fluorescence, stomatal conductance, ABA and free proline levels. Besides these parameters, the wooden box screening for drought tolerance at the seedling stage and delayed leaf senescence could be interesting with respect to evaluating and determining drought tolerance. Physiological, biochemical and agronomic responses to water deficit at the flowering stage of cowpea detected an increase of canopy temperature and proline content, as well as a decrease of gaseous exchanges and starch content, that eventually affected the yield components with the exception of seed number per pod.<sup>139</sup>

The knowledge transfer between plant species and cultivars should be taken with care because differences in drought tolerance were detected when evaluating distinct plant species or cultivars. For example, a comparison of physiological responses to drought between *V. unguiculata* and *Phaseolus vulgaris* demonstrated that both species significantly differ in the responses evaluated by leaf gas exchange parameters.<sup>140</sup>

**Table 3.** Genes identified as being involved in drought tolerance in cowpea

Gene designation	Code number	Gene function	Author
<i>CPRD8</i>	D83970	Response to dehydration stress	luchi <i>et al.</i> (1996) <sup>142</sup>
<i>CPRD14</i>	D83971	Response to dehydration stress	luchi <i>et al.</i> (1996) <sup>142</sup>
<i>CPRD22</i>	D83972	Response to dehydration stress	luchi <i>et al.</i> (1996) <sup>142</sup>
<i>CPRD12</i>	D88121	Response to dehydration stress	luchi <i>et al.</i> (1996) <sup>143</sup>
<i>CPRD46</i>	D88122	Neoxanthin cleavage enzyme involved in ABA biosynthesis	luchi <i>et al.</i> (1996) <sup>143</sup>
<i>VuNCED1</i>	AB030293	9-Cis-epoxycarotenoid dioxygenase involved in a key step of ABA biosynthesis	luchi <i>et al.</i> (2000) <sup>132</sup>
<i>VuABA1</i>	AB030295	Zeaxanthin epoxidase involved in early step of ABA biosynthesis	luchi <i>et al.</i> (2000) <sup>132</sup>
<i>VuPLD1</i>	U92656	Putative phospholipase D, a major lipid-degrading enzyme in plant	El-Maarouf <i>et al.</i> (1999) <sup>135</sup>
<i>VuPAP-α</i>	AF165891	Putative phosphatidate phosphatase, important for the enzymatic cascade leading to membrane lipid degradation under environmental stresses or senescence	Marcel <i>et al.</i> (2000) <sup>145</sup>
<i>VuPAP-β</i>	AF171230	Putative phosphatidate phosphatase, important for the enzymatic cascade leading to membrane lipid degradation under environmental stresses or senescence	Marcel <i>et al.</i> (2000) <sup>145</sup>
<i>VuPAT1</i>	AF193067	Galactolipid acyl hydrolase involved in membrane degradation induced by drought stress	Matos <i>et al.</i> (2001) <sup>136</sup>
<i>VuC1</i>	AF278573	Protein inhibitor of cysteine proteinase belonging to the papain family	Diop <i>et al.</i> (2004) <sup>146</sup>
<i>dtGR</i>	DQ267474	Dual-targeted glutathione reductase, a key enzyme involved in detoxification of AOS	Contour-Ansel <i>et al.</i> (2006) <sup>138</sup>
<i>cGR</i>	DQ267475	Cytosolic glutathione reductase, a key enzyme involved in detoxification of AOS	Contour-Ansel <i>et al.</i> (2006) <sup>138</sup>
<i>VucAPX</i>	U61379	Cytosolic ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta <i>et al.</i> (2006) <sup>111</sup>
<i>VupAPX</i>	AY466858	Peroxisomal ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta <i>et al.</i> (2006) <sup>111</sup>
<i>VusAPX</i>	AY484493	Stromatic ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta <i>et al.</i> (2006) <sup>111</sup>
<i>VutAPX</i>	AY484492	Thylakoidal ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta <i>et al.</i> (2006) <sup>111</sup>
<i>GST</i>		Glutathione-S-transferase, a well-recognized stress-related gene	Gazendam and Oelofse (2007) <sup>148</sup>
<i>PR-1</i>		Pathogenesis-related-protein-1, a well-recognized stress-related gene	Gazendam and Oelofse (2007) <sup>148</sup>
<i>VuNSR4</i>	ABA55727.1	Digalactosyl diacylglycerol sintase 1	Silva <i>et al.</i> (2012) <sup>149</sup>
<i>VuNSR10</i>	AAC49405.1	Kinase protein calcium dependent	Silva <i>et al.</i> (2012) <sup>149</sup>
<i>VuNSR44</i>	BAA13541.1 BAA12161.1	CPRD12 protein CPRD12 protein	Silva <i>et al.</i> (2012) <sup>149</sup>
<i>VuNSR47</i>	BAA12160.1	CPRD8 protein ('old yellow' enzyme)	Silva <i>et al.</i> (2012) <sup>149</sup>
<i>VuNSR49</i>	BAB11932.1	CPRD65 protein	Silva <i>et al.</i> (2012) <sup>149</sup>

### Drought tolerance genes

Transcriptomic studies have been developed to identify genes, pathways and processes important in controlling plant response to multiple abiotic or biotic stresses, thus providing candidate targets for stress tolerance improvement.<sup>141</sup> Many cowpea drought-related genes have been deduced from previously recognized candidate genes for drought tolerance in other related species, and were subsequently confirmed by their differential expression in drought-stressed versus non-stressed cowpea plants. On the other hand, studies of the differential expression of cowpea genes in experimental plants subjected to different levels of water privation have led to the identification of cowpea genes involved in drought responses.<sup>112</sup>

Many cowpea genes are now recognized as being involved in drought responses (Table 3). Using a differential screening method, luchi *et al.*<sup>142</sup> isolated 24 cDNA clones that corresponded to dehydration-induced genes from a cowpea variety (IT84S-2246-4) displaying a high drought tolerance. These cDNA clones represented ten different genes, nine of which were specifically induced by dehydration stress. Five of these drought-associated genes were characterized further (*CPRD8*, *CPRD14*, *CPRD22*, *CPRD12* and *CPRD46*),<sup>142,143</sup> followed by a description of two additional drought-inducible genes all from the same cowpea variety (*VuNCED1* and *VuABA1*).<sup>132</sup> *VuNCED1* encodes a 9-cis-epoxycarotenoid dioxygenase that catalyses a key step in ABA biosynthesis, whereas *VuABA1* encodes a zeaxanthin epoxidase<sup>132</sup> involved in another important key step of ABA

biosynthesis. Indeed, zeaxanthin epoxidase has been reported as being required for resistance to osmotic and drought stress, ABA-dependent stomatal closure and regulation of the expression of stress-responsive genes.<sup>144</sup>

According to the degradation of membrane lipids that occur under drought stress conditions,<sup>134</sup> several other cowpea drought-related genes are recognized to be involved on lipid metabolism. El-Maarouf *et al.*<sup>135</sup> isolated and characterized the cowpea *VuPLD1* gene that encodes a phospholipase D, which is the main enzyme responsible for the drought-induced degradation of membrane phospholipids. In a drought stress susceptible cultivar, phospholipase D activity and *VuPLD1* expression were highly stimulated by drought stress, whereas they remained unchanged in a tolerant cultivar.<sup>135</sup> From the leaves of the same cultivars, Matos *et al.*<sup>136</sup> isolated a *VuPAT1* (putative patatin-like) gene that encodes for galactolipid acyl hydrolase. A rapid increase of *VuPAT1* expression was also observed in the susceptible cultivar under drought conditions, whereas the tolerant exhibited lower levels of transcripts. These results suggest that drought stress in cowpea stimulates the hydrolysis of galactolipids, which are the main components of chloroplast membrane. *VuPAP- $\alpha$*  and *VuPAP- $\beta$*  are two cDNAs encoding putative phosphatidate phosphatases (PAPs) that were cloned from cowpea leaves by Marcel *et al.*<sup>145</sup> PAPs play a role in the enzymatic cascade that leads to membrane lipid degradation under environmental stresses or senescence.<sup>137</sup> Marcel *et al.*<sup>145</sup> revealed that gene expression of *VuPAP- $\alpha$*  remained very low during drought treatments, being strongly stimulated after rehydration. On the other hand, *VuPAP- $\beta$*  expression did not vary in plants submitted to water stress by withholding irrigation, although it increased rapidly in air desiccated leaves.

Metabolic and adaptive processes, in which the adaptation to drought stress is included, comprise the regulation of protein degradation via the use of protease-specific inhibitors<sup>146</sup> and cellular protection against oxidative damage through the regulation of anti-oxidant enzymes and free radical scavengers.<sup>147</sup> The expression of cowpea cystatin (cowpea leaf protease inhibitor; *VuCl*) gene, evaluated at mRNA (Northern analysis) and protein (Western analysis) levels, suggested that two cystatin transcripts producing two distinct polypeptides would lead to a multiplicity of forms related to multiple biological roles.<sup>146</sup>

A noticeable activation of cowpea antioxidant metabolism has been detected under progressive water stress by studying drought-related genes. The cloning and sequencing of two new cDNAs encoding a putative dual-targeted (*dtGR*) and a cytosolic (*cGR*) GR from cowpea leaves was performed by Contour-Ansel *et al.*<sup>138</sup> The expression of both genes in cowpea leaves of drought-sensitive and drought-tolerant plants subjected to different drought stress conditions revealed that up-regulation of *cGR* expression is directly related to the intensity of stress in both cultivars, although *dtGR* expression was different in susceptible and resistant cultivars. The results revealed the participation of GR in drought responses of both cowpea cultivars, which, in susceptible cultivar, involves both GR genes.<sup>138</sup> The expression of other antioxidant enzyme genes (ascorbate peroxidases; APX) was also studied in the cowpea response to progressive drought, rapid desiccation and application of exogenous ABA. Four new cowpea cDNAs encoding putative cytosolic (*VucAPX*), peroxisomal (*VupAPX*), chloroplastic (stromatic *VusAPX*) and thylakoidal (*VutAPX*) ascorbate peroxidases were isolated and characterized.<sup>111</sup> When the expression levels of *VucAPX* and *VupAPX* were followed in drought-tolerant and sensitive cultivars, an increase in steady-state transcripts levels was observed

in response to rapid water loss and exogenous ABA treatment in drought-sensitive cultivar, whereas no significant changes in drought-tolerant cultivar were registered. Also, the *VusAPX* gene expression was strongly stimulated at low levels of water stress in drought-tolerant cultivar. The higher expression of all these genes in tolerant cultivars, compared to sensitive ones, again suggested that cowpea is a drought-tolerant species compared to other crops, indicating that even the more sensitive cultivars have some level of resistance to water deficits.<sup>111</sup> Two other well-recognized stress-related genes, *GST* (glutathione-S-transferase) and *PR-1* (pathogenesis-related-protein-1), were identified in cowpea by suppression subtractive hybridization (SSH) using drought-tolerant and susceptible lines.<sup>148</sup> Silva *et al.*<sup>149</sup> followed the effect of drought and heat stresses on cowpea nodules by evaluating the differential gene expression, using a cDNA-AFLP approach, and identified 14 differentially expressed nodule stress responsive genes. These genes are involved in different metabolic processes, five (*VuNSR4*, *VuNSR10*, *VuNSR44*, *VuNSR47* and *VuNSR49*) of which were related with the nodule protection under abiotic stress conditions as revealed by their expression levels.<sup>149</sup>

### MicroRNA drought regulation

MicroRNAs (miRNAs) regulate gene expression at the post-transcriptional level through the recognition of target RNAs by almost perfect base complementary. Several functional analyses have demonstrated that miRNAs are involved in a variety of plant developmental processes and play important roles in plant resistance to abiotic and biotic stresses.<sup>150,151</sup> From two cowpea genotypes, one drought-tolerant and another drought-sensitive, 157 miRNAs were identified, 44 of which were drought-associated, with 30 being upregulated and 14 downregulated in drought conditions. Cowpea miRNAs from leaves and roots of plants subjected to drought treatment were also identified and validated by a real-time-quantitative polymerase chain reaction.<sup>152</sup> The results demonstrated that the same miRNAs in different tissues respond differently to drought stress. Both studies suggest that miRNAs could play an important role in cowpea response to drought stress by regulating the expression levels of drought-related genes.

## CONCLUSIONS

Global climate changes have an enormous impact on plant diversity patterns with significant current negative effects. In Europe, it is the Mediterranean countries where a higher impact of climate changes is expected, including an increase in drought, high temperatures and water scarcity. Drought is a critical constraint for agricultural production yield, which is currently expanding worldwide and affecting an increased number of countries. New strategies are thus required to overcome this major challenge in agricultural production systems, such as the development of new farming systems and the use of undervalued crop varieties. As a result of its natural tolerance to water scarcity conditions and high temperatures, cowpea could be considered as a valued crop for increasingly drought scenarios. Besides drought tolerance, cowpea also presents high levels of protein and the capacity to establish symbiotic associations with distinct microorganisms (mainly rhizobia and mycorrhizal fungi) that turn it into an environmentally friendly crop. This legume could also be a useful plant model for understanding the mechanisms involved in drought tolerance. The existence of several cowpea varieties and cultivars, displaying different tolerance levels to drought conditions, provides an



excellent germplasm resource for identifying new candidate genes involved in the responses to drought stress tolerance and also for use in future breeding programmes. DNA molecular markers have shown to be a good tool for germplasm evaluation and the selection of the most interesting drought stress/tolerant genotypes. Because MAS can facilitate the selection of elite germplasm and accelerate plant breeding programs, the identification of the precise position of drought-related known genes and of new candidate genes should be carried out. Integration of data from phenotype, biochemical and molecular characterization will help to clarify the resilience and resistance of cowpea under drought and provide sufficient cowpea knowledge for the development of drought-tolerant varieties. For these reasons, cowpea can also be an important plant model for the development of other crop varieties that are more drought tolerant.

## ACKNOWLEDGEMENTS

This work is supported by European Investment Funds by FEDER/COMPETE/POCI – Operacional Competitiveness and Internacionalization Programme, under Project POCI-01-0145-FEDER-006958 and National Funds by FCT – Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2013.

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