

Introduction
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In order to efficiently utilize plant genetic resources and to have effective conservation and management of these resources, an adequate knowledge of existing genetic diversity is of fundamental interest. The improvement of crop genetic resources has largely been dependent on modern plant breeding techniques; however, the introduction of material from wild relatives and local varieties is gaining in popularity. This shift in breeding technique in a modern commercial setting is in response to the narrow genetic range of many crop species. This narrow genetic diversity hinders the ability to adequately respond to changing climate and commercial needs in a timely fashion. At the genetic level, natural populations possess high variability (Ahmed, et al.

, 2011, Ballian, et al., 2012, Snoeijs and Potapova, 1998), which enables these populations to adapt rapidly to variable environmental pressures; and it is this variability, that may be useful to modern plant breeding systems.

Modern plant breeding depends on the assessment and manipulation of genetic diversity to generate highly productive and resistant varieties. The genetic diversity, i. e. differing allele frequencies, within genes of crop plant wild relatives have the potential to reintroduce useful characteristics that may have been lost during the domestication process. This approach has been used with great success for example in rice (Bimpong, et al., 2011, McCouch, et al.

, 2012), and in oil seed rape (Johnston, 1974). In tomato allelic differences at the fw2.2 locus have been implicated in the increase of fruit size during the domestication process (Frary, et al., 2000). In strawberry varieties, a SNP

variant change in a MYB10 gene has been shown to control fruit colour (Hawkins, et al.

, 2016). In *Arabidopsis* significant SNP relating to salt stress have been identified (Negrao, et al., 2013); and in soybean two cell division genes with insertion/deletion variants have been implicated in short root phenotypes in *Glycine soja* as compared with cultivated soybean (Prince, et al., 2015).

It is likely therefore, that any potential genetic diversity seen within red clover ecotypes could be used to introgress useful traits from its wild relatives into current breeding lines. Red clover is a relatively newly domesticated species, and it was not until around the year 1,000 AD that it was first intentionally domesticated in Southern Spain (Kjærsgaard, 2003). Modern varieties are extensively used as silage due to the growth habit. Many of the varieties currently on the market in Europe originate from a Mattenkleef type of plant, with their growth habit characterised by tall upright stems. However, natural populations have been reported to show varied growth habits, characteristically with less dependency on the central crown and a more prostrate nature to stem growth.

Under certain temperature and moisture conditions, stems of these prostrate communities are able to produce nodal root growth (Frame). Potential genes underlying the prostrate growth habit of some red clover ecotypes, as well as the latent disease resistance often associated with natural populations, could be of considerable use to the IBERS breeding program, in terms of furthering the range of climatic and agricultural conditions suited to red clover growth and use. When plants spread into new ecosystems they may encounter new

and diverse sets of selective pressures (Perez, et al., 2006, Vannier, 2009), which may lead to changes in growth habit, and morphology and the partitioning of metabolites and disease resistance. This natural diversity in growth habit has enabled red clover ecotypes to adapt to the varied environmental conditions in which it exists (Taylor and Quesenberry, 1996). The genetic diversity of wild and cultivated populations of red clover have previously been studied using low throughput methods of isoenzyme (Mosjidis, et al., 2004), AFLP (Collins, et al.

, 2012), RAPD (Dias, et al., 2008) (Dias, et al., 2008) and SSR markers (Ahsyee, et al.

, 2014). It is now possible to study large scale genome wide variation in populations by using next generation sequencing (NGS) technologies. Genotyping by sequencing (GBS) (Elshire, et al., 2011) has become a popular NGS technique to identify large scale variation both with and without a reference genome. The use of GBS has allowed researchers to identify thousands to millions of single nucleotide polymorphism (SNP) markers, which can be used to identify genetic variation within and between study populations. These SNP can be used in the analysis and dissection of complex traits, especially those involved in adaptive selection. We report here on the genotyping by GBS of 70 wild populations of red clover from Europe and Asia and five European elite breeding lines from the seed bank at IBERS. There has been no previous characterization of the genetic resources of wild ecotypes and landraces held at IBERS.

Therefore, the breeders have not been enthusiastic to exploit this diversity, and the pattern of genetic variation between and within these ecotypes has remained unknown. We aligned the GBS data to the red clover reference genome (De Vega, et al., 2015). We found a four group population structure using Cluster and nine potential ancestry groups using STRUCTURE.

There was moderate genetic diversity between the groups; and a correlation of genotype to geography and SNP that correlated to adaptation to their geography; and from a genome wide association study (GWAS) we identified possible SNP involved in flowering time.