



Review

The Role of Crop Wild Relatives and Landraces of Forage Legumes in Pre-Breeding as a Response to Climate Change

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Abstract: Climate change and the variable extreme weather conditions that today's agricultural producers are dealing with represent some of the most important issues in food production. Nowadays, weather patterns are increasingly unpredictable, characterized by frequent fluctuations in temperature, precipitation, and extreme weather events. As a result, there is an increasing demand for scientists to develop more resilient and tolerant crop cultivars. Plant breeders must become creative and utilize all available resources to create modern high-yielding and widely adapted cultivars to help agriculture grow and thrive amidst the emerging changes. Forage legumes, due to their beneficial characteristics, are among the crops that can contribute to mitigating the consequences of climate change. Furthermore, what certainly does not contribute to weather conditions is the erosion of plant genetic material, which has been caused by the modernization of agriculture and the selection of the best cultivars with desirable traits over many years. Crop wild relatives (CWRs) and landraces represent plant genetic materials rich in novel gene variants that contain traits for resistance and tolerance to different climatic conditions. To expand the genetic base of cultivars and mitigate the consequences of climate change, breeders are increasingly utilizing pre-breeding methods. These methods include all the activities connected to the identification of desirable genes and traits from un-adapted materials, such as CWRs, and the transformation of these traits to an intermediate set of materials that can be used for creating new cultivars. This review paper will cover the pre-breeding process, including its components, and the resistance and/or tolerance of the CWRs and landraces of forage legumes to different extreme environmental conditions.

Keywords: legumes; extreme conditions; plant genetic resources; desirable traits; molecular markers; QTL



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1. Introduction

Human population growth is expected to increase by 33% until 2050, which means that the need for agricultural production will increase by 70% over the same period due to improved living standards [1]. Global meat consumption will increase by 15% and milk demand by 35% by the year 2030, mainly due to population growth [2,3]. In that case, the demand for fodder will increase, and farmers who are already producing voluminous fodder will obtain an opportunity on the market [4]. As part of the Common Agricultural Policy (CAP) of the European Union, the Agri-Environmental Schemes (AES) were created to reduce the intensification of agriculture and its harmful impact on the environment through voluntary contracts with farmers that are financially supported for

their implementation of green measures that benefit biological diversity and soil conservation. Sowing legumes is often proposed as an AES within CAP [5]. Examples of such voluminous crops are alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*), which are highly nutritious perennial voluminous forages for animal feed that have a high content of protein, especially alfalfa [6–9]. Food production is limited by non-renewable energy resources such as soil, and its impact on ecosystems needs to be reduced [10]. Therefore, the production of legumes provides a good alternative for soil recovery, considering that they are nitro-fixing plants and can increase the rhizosphere of the soil by their roots. Along with the increasing demand for agricultural products, climate change represents an additional challenge to everyday crop production [11,12]. This means that abiotic and biotic stresses lead to difficult growth and development of the crop, which results in a loss of productivity and quality if the plants are exposed to them for a long time [13]. In other words, yields are negatively affected by several factors such as variable and extreme climatic conditions (drought, heat waves, excess floods, low temperatures), pathogens, and pests [14,15], which result in different metabolic and physiological plant response [16]. This review will cover plant tolerance and/or resistance to different abiotic stresses from recent research. According to Galluzzi et al. [17], scientists were asked about their perception of weather events from the year 2015 to the year 2020, and most of them agreed on irregular drought periods and rainfall patterns. If legumes are grown, like alfalfa and clover, they can increase soil biodiversity and soil carbon content, improve pest control and soil fertility, reduce soil erosion and greenhouse gas emissions, and fix atmospheric nitrogen [5,18,19]. These are all measures to mitigate climate change, but the creation of new cultivars that are resistant and/or tolerant to the mentioned abiotic stresses is the measure that this review paper will be about. The creation of new cultivars can be achieved by using crop wild relatives (CWRs) and landraces as sources of alleles that are novel gene variants for complex adaptive traits [20]. CWRs are wild plant species that are genetically related to cultivated species but, unlike high-yielding cultivars, they contain a wide range of traits due to the growth of populations in the natural habitat within local environmental conditions [21]. It is important to highlight the value of CWRs, especially in these times when the world is faced with extreme climate conditions that occur more frequently and in severity than ever before [22]. To genetically enrich high-yielding cultivars with useful and desirable traits and genes, CWRs and landraces are used in pre-breeding systems [23,24]. Pre-breeding is a system for the identification of useful traits and genes from un-adapted wild plant material/germplasm and the transformation of these traits to an intermediate set of materials that breeders can further use to develop new cultivars with a broader range of genetic bases [25–27]. The knowledge gap becomes evident when considering the significantly higher yields of cultivars produced under controlled conditions versus the lower yields obtained in the field. This can be attributed to the intense and more frequent extreme climate variations, leading to plant stress that affects their genetic yield potential. The aim of this review is to introduce novel concepts and methodologies such as pre-breeding for developing new high-yielding crop varieties, and to shed light on the potential of CWRs and landraces, as well as the current state of research worldwide. Moreover, it aims to provide insights, based on recent research, into expediting the characterization and identification of CWRs and landraces through the use of molecular markers and field research methods. Pre-breeding characterization and the evaluation of CWRs and landraces are essential. This process facilitates faster trait identification, utilization, and integration into elite cultivars.

2. Crop Wild Relatives of the Most Widespread Perennial Forage Legumes

According to Purugganan [28], domestication of wild plant species as a source of food (nutrients and fiber) started 12,000 years ago, and because of that long process, the human population has survived until today. As a result of long-term domestication and cultivation as well as market demand for high-yielding and resilient crops, the focus of breeders has become directed toward a small number of cultivars and species instead of locally adapted

and genetically diverse crops as previously grown [29]. While domestication has enabled the spread of crop cultivation over wider geographical areas and longer periods [30], systematic breeding has led to a 75% loss of plant genetic resources (PGRs) and a 90% loss of crop cultivars [31,32]. According to Humphries et al. [33], cultivated alfalfa has lost between 9 and 30% of its genetic diversity when compared to wild populations. This consequence of systematic breeding can be prevented by using CWRs and landraces, thereby expanding the already narrowed genetic base with useful genetic variations [34]. Wild species, unlike domesticated ones, were not subjected to strict anthropogenic selection pressure, but through evolution, they were exposed and adapted to extreme and changing weather conditions [35]. In addition to alfalfa and clover, the pre-breeding program can also be used for other crops such as wheat [36], barley [37], maize [38], soybean [39], tomato, etc. [40]. The Fabaceae family has over 19,500 species which makes it the third largest family when considering flowering plants [41], and there is a large selection of CWRs that can be used in the pre-breeding of alfalfa and clover. Therefore, due to their diverse genetic pool CWRs can grow in environments that would cause great stress for modern cultivars. They have different levels of resilience to different stresses depending on their origin [42]. For example, to achieve adapted genotypes to abiotic stress such as drought and achieve outstanding production, introducing drought-resistant alleles into elite cultivars can be considered [43].

2.1. Alfalfa

According to Inostroza et al. [44] and Şakiroğlu and İlhan [45], the primary gene pool for the introduction of desirable properties in alfalfa is located in the *Medicago sativa*–*falcata* complex, which includes the following subspecies: *M. sativa* subsp. *caerulea* (2n), *M. sativa* subsp. *falcata* (2n and 4n), *M. sativa* subsp. × *hemicycla* (2n), *M. sativa* subsp. *glutinosa* (2n), *M. sativa* subsp. *sativa* (4n), *M. sativa* subsp. × *varia* (4n), and *M. sativa* subsp. *glomerata* (4n). Within the *M. sativa* complex, *M. sativa* subsp. *caerulea* and *falcata* represent a valuable CWR, a potential source of germplasm that contains the trait of drought resistance. *M. sativa* subs. *falcata* (also a donor for cold tolerance) hybridizes well at the diploid level with *M. sativa* subsp. *caerulea* (drought tolerance and winter hardiness), while at the tetraploid level, it hybridizes with the wild-growing, purple-flowered tetraploid *M. sativa* [33]. Yin et al. [46] conducted research where they determined the expansion of multiple gene and transcription factor families (such as SOS homologous genes, NAC, C2H2, and CAMTA) that could be the reason for the ability of *Medicago ruthenica* to tolerate drought, salinity, alkalinity, and cold-snowy winters. Similar results were obtained by Wang et al. [47] who used comparative genomics and transcriptomic analyses to elucidate the mechanisms of stress resistance in *M. ruthenica*. Their results showed that the extended FHY3/FAR1 family participates in drought resistance and that many genes responsible for the tolerance to environmental stresses remained in *M. ruthenica* compared to other *Medicago* species. In addition to the resistance of CWR to drought, numerous studies of tolerance to salinity or even the cold have been carried out. In the research conducted by Guan et al. [48], *M. ruthenica* tolerated salinity (concentration: 0, 50, 100, 150, 200, or 250 mM NaCl) and alkalinity (concentration: 1, 5, 10, 15, 20 or 25 mM Na₂CO₃) well, which means that it can be used in environments of different acidity. El-Shafey and Al-Sharif [49] conducted a study in which they examined the germination of CWR seeds under saline conditions, and their study showed that the germination percentage of *M. sativa* and *Medicago lupulina* did not change significantly in most saline levels compared to the control. In a study conducted by Sarri et al. [50], where *Medicago arborea*, *M. sativa*, and their hybrid Alborea were compared for salinity tolerance, in all three salinity treatments, *M. arborea* showed greater growth stability and salinity tolerance. Pennycooke et al. [51] did a comparative analysis of *Medicago truncatula* and *M. sativa* subsp. *falcata* to determine which one could acclimate to the cold and the results showed that *M. truncatula* survived at −4 °C but did not acclimate to the cold, while *M. falcata* acclimated to cold and survived at −14 °C.

2.2. Clover

Like the genus *Medicago*, some types of clover also have traits of tolerance and/or resistance to various environmental stresses. Can et al. [52] carried out a study to determine how salinity affects germination, plant growth, and development of strawberry clover *Trifolium fragiferum*, and they concluded that it has good salinity tolerance. Another study showed that CWR *T. fragiferum* is more tolerant to floods/waterlogging than other wild accessions and the control cultivar Palestine used in the trial [53]. In the research conducted by Jēkabsone et al. [54], the salt tolerance of several CWR *T. fragiferum* from environments with different salinity levels (TF1-TF4 and TF7-TF9) was evaluated under controlled conditions. Their results showed that samples collected from Lake Liepājas (TF1) had the highest salinity tolerance (also the highest tolerance to waterlogging) with the highest biomass production capacity among all other wild accessions. Research conducted by Dūmiņš et al. [55] while using coexisting species, wild *T. fragiferum* and *Trifolium repens*, in salinity treatments and rhizobial symbiosis showed good growth of *T. fragiferum* in salinity treatment when coexisting with *T. repens*, along with the fact that the effect of the bacterial inoculant was more pronounced. They concluded that *T. fragiferum* has greater physiological salinity tolerance than *T. repens*. Furthermore, in addition to the tolerance of clover to salinity and flooding, scientists also investigated tolerance to the cold, freezing, and drought. In the research conducted by Zanotto et al. [56], 19 landraces, 2 semi-wild, and 27 wild *T. pratense* accessions were used for the testing of winter survival and freezing tolerance to detect useful variations and thus improve the cultivated Nordic red clover. In their study, most of the gene bank accessions outperformed the commercial cultivars in winter survival and freezing tolerance and therefore may be a genetic resource for future improvement in these traits. Zanotto et al. [57], in their study, tested 10 wild/landrace specimens for freezing tolerance, with the landrace specimen Hyrkās ME0101 surviving up to $-13.09\text{ }^{\circ}\text{C}$ and the wild accession Raunavaara AK0402 surviving up to $-12.42\text{ }^{\circ}\text{C}$, which makes them both freezing tolerant. In the research conducted by Abbasi et al. [58], the drought resistance of 15 wild and 2 cultivated annual accessions was tested. They concluded that in Mashhad, Iran, in descending order, *Trifolium purpureum*, *T. echinatum*, *T. hirtum*, *T. diffusum*, *T. lappaceum*, and *T. resupinatum* (wild) produced the highest rates of forage and showed a high resistance to a water deficit compared to other accessions and cultivars, while in Urmia, Iran, the order was *T. resupinatum* (cultivar), *T. echinatum*, *T. lappaceum*, *T. purpureum*, and *T. diffusum*; therefore, they concluded that the cultivated *Trifolium* species (*T. resupinatum*) is more suitable for humid temperate regions with high rainfall such as Urmia region. In Table 1, there is a list of different *Medicago* and *Trifolium* species (wild or landrace) resistant or tolerant to different stresses.

Table 1. Crop wild relatives and landraces of alfalfa and clover that are resistant or tolerant to different types of stress.

Genus	Species	Germplasm	Tolerance to Stress	Authors
<i>Medicago</i>	<i>M. sativa</i>	Wild	Drought	[59]
	<i>M. arborea</i>	Wild	Salt, drought	[50,60]
	<i>M. laciniata</i>	Wild	Drought	[61]
	<i>M. ciliaris</i>	Wild	Drought	[62]
	<i>M. intertexta</i>	Wild	Salt	[49]
	<i>M. sativa</i> cv. Longzhong	Landrace	Drought	[63]
	<i>M. ruthenica</i>	Wild	Drought, cold, salt	[46–48]
	<i>M. polymorpha</i>	Wild	Drought	[64]
	<i>M. truncatula</i>	Wild	Drought, cold	[65,66]
	<i>M. falcata</i>	Wild	Drought, cold, salt, alkalinity	[67–69]

Table 1. Cont.

Genus	Species	Germplasm	Tolerance to Stress	Authors
Trifolium	<i>T. fragiferum</i>	Wild	Salt, flood	[53–55,70]
	<i>T. pratense</i> var. <i>pratense</i> Hyrkäs me0101	Landrace	Cold	[57]
	<i>T. pratense</i>	Wild, landrace	Drought, salt, freezing	[56,71–73]
	<i>T. purpureum</i>	Wild	Drought	[58]
	<i>T. isthmocarpum</i>	Wild	Salt	[74]
	<i>T. subterraneum</i>	Wild	Waterlogging	[75]
	<i>T. michelianum</i>	Wild	Waterlogging	[76]

3. Pre-Breeding

Pre-breeding is a link between genetic resources and breeding programs [77]. It is a long-term process whose goal is not to create new cultivars or hybrids, but to create germplasm that can be included in the breeding program to obtain new genotypes [78]. It can be used as a system to accelerate the preparation of CWR and landrace material for the introduction of such material into breeding programs [79]. According to Indu et al. [15], introducing CWRs through conventional breeding approaches is complicated because of linkage drag and small chances of getting fertile seeds and robust plants. For that reason, new approaches such as pre-breeding are necessary.

Several factors that represent an obstacle to pre-breeding, according to Sharma et al., [80] are a large amount of germplasm, a lack of information on traits of great economic importance, and the preference of breeders for working collections. The main obstacles, according to Annicchiarico et al. [81], are non-desirable traits that some wild populations carry such as low forage yield potential, prostrate growth habit, and possible diploidy. Regardless of the mentioned disadvantages, the advantages of pre-breeding using CWRs and landraces are much greater. Not only can they significantly expand the source of genetic variation and selection for yield, tolerance, resistance, and improved crop quality, but they can also contribute to the world economy, where the annual contribution (economic returns to agricultural industries worldwide) was estimated at USD 186.3 billion in 2020 [82]. Another interesting and useful way of using CWRs is speed breeding, where it is possible to produce several generations in one year in facilities with controlled conditions while enabling selection against undesirable traits and achieving stable genetic material of plants [83].

The pre-breeding process (Figure 1) starts with collecting various plant genetic resources, including cultivated types of germplasm, landraces, exotic germplasm, and crop wild relatives. Promising germplasms that contain desirable traits are identified through the evaluation and characterization of genetic resources by setting up field experiments and nurseries (Figure 2). That is followed by crossing the identified genetic resources with a widely adapted cultivar, which results in the establishment of a large pre-breeding population with desirable agronomic traits. For many years, the scientific research community has felt the need for more intensive work in the field of breeding, therefore many scientists have joined various projects to contribute to the work with CWRs and landraces. Some projects, such as “Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives,” aimed to enhance capacities in developing countries and focused on the pre-breeding of 19 important crops for food security. As part of this project, 17 papers on 13 crops were published, including 1 on alfalfa [20]. Projects like Farmer’s Pride (<https://more.bham.ac.uk/farmerspride/>, accessed on 18 June 2024) and the SADC Crop Wild Relatives (<http://www.cropwildrelatives.org/sadc-cwr-net/>, accessed on 18 June 2024) have contributed to the development of tools aimed at planning for the conservation of wild crops, such as wild alfalfa and red clover in Europe and Africa [84].

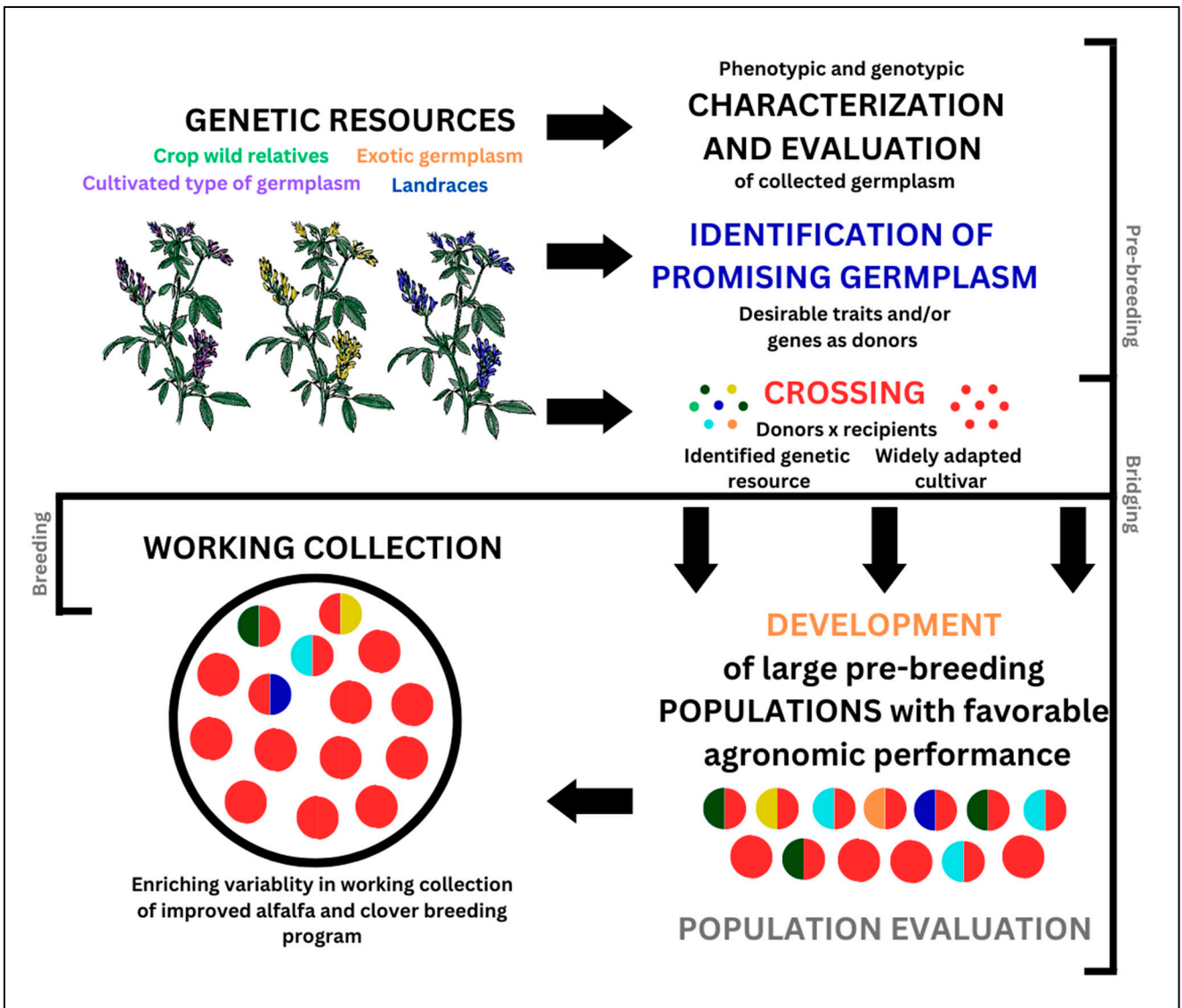


Figure 1. The process of pre-breeding as a “bridge” between genetic resources (gene banks) and breeding programs.

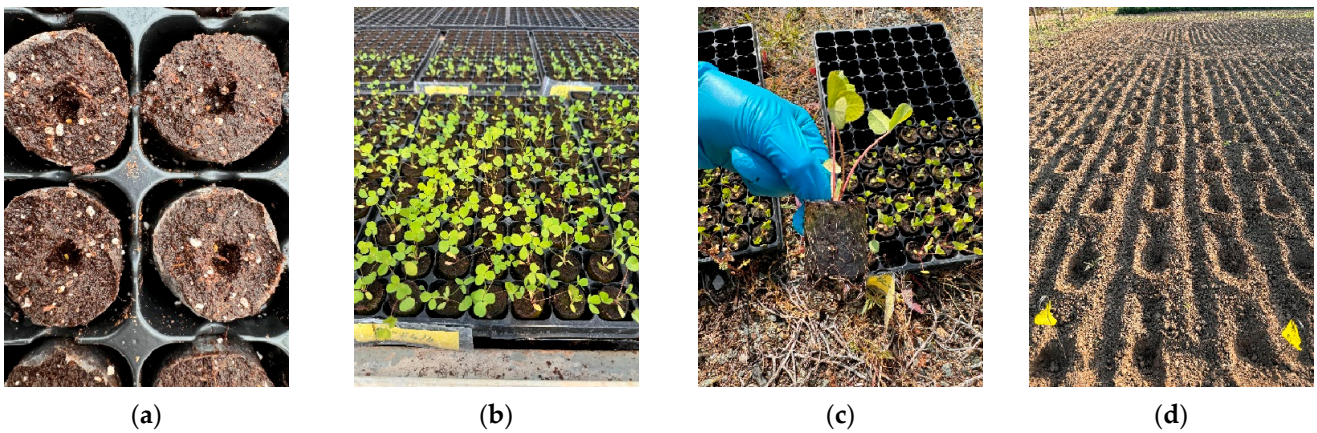


Figure 2. Cont.

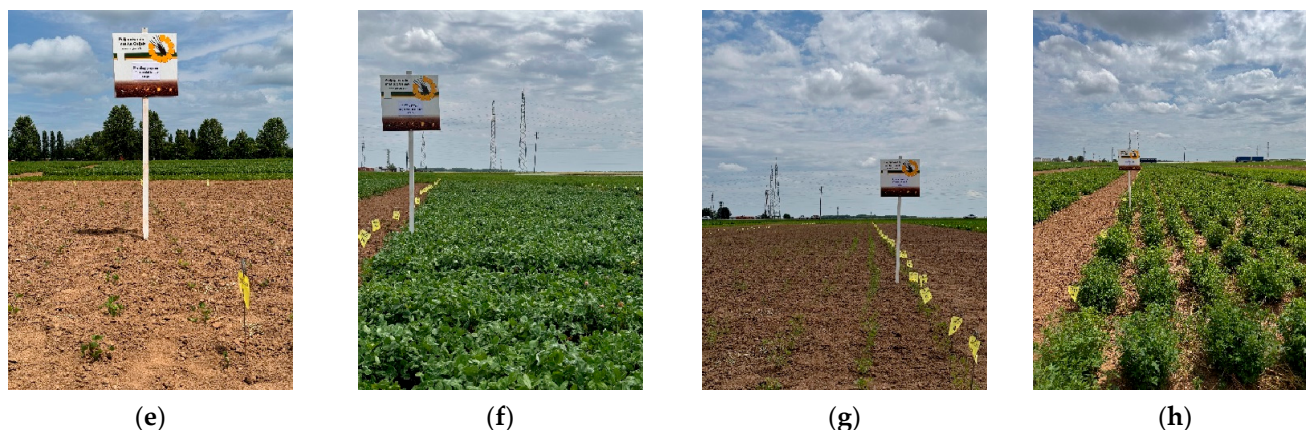


Figure 2. Establishment of alfalfa and red clover breeding nurseries and testing of materials in multi-year field experiments at Agricultural Institute Osijek, Croatia: (a) sowing the plant seed materials of alfalfa and red clover in peat pots; (b) plant growth of alfalfa under controlled greenhouse conditions; (c) transferring of individual young plants of the gene collection of alfalfa from greenhouse to field; (d) soil preparation for growing individual plants in the space plant nursery; (e) space plant nursery of red clover established in the year 2024; (f) red clover variety experiment established in the year of 2023; (g) space plant nursery of alfalfa established in the year 2024; and (h) space plant nursery of alfalfa established in the year 2023.

3.1. Genetic Resources

Concerning climate change in the world, public and private plant breeders are working on new, more competitive cultivars on the market, and to achieve that, they need diverse plant genetic resources—PGR [85]. According to Dulloo et al. [86], no nation is self-sufficient in plant genetic resources and all nations must have access to germplasm originating from other countries and regions. Therefore, PGRs are mostly kept in 1625 gene banks all over the world [87]. They collect CWRs and other plant materials that have traits of interest to breeders, so their goal is not only to collect and preserve diverse germplasm but also to provide resources to breeders [88]. Various PGRs can be found in gene banks such as CWRs [89], landraces [90], cultivated-type germplasms [91], and exotic germplasms [92]. According to Karam et al. [64], the genus *Medicago* includes approximately 85 species and 18 infraspecies of which 2 are shrubs, 20 herbaceous perennials, and 63 herbaceous annuals. According to Căntar and Dincă [93], there are approximately 300 species of the genus *Trifolium* in the world, annual and perennial. The global germplasm collection comprises 651,024 forage accessions, with 35% being wild species, 13% domestic species, 4% advanced varieties, 3% breeding materials, and 45% others [94]. Most of the accessions found in gene banks have only basic passport data, because collecting data on traits of interest is very expensive, labor-intensive, and requires multiple locations and years for evaluation [95]. For this reason, breeders sometimes need to do characterization and evaluation of accessions by themselves. The traits of interest for alfalfa and clover breeders are yield, nutritive value, persistence, disease and pest resistance, winter hardiness, frost tolerance, drought, salt tolerance, etc. [96]. There are many CWRs and landraces with different traits of interests for breeders so the necessity for their characterization and evaluation is obligatory. The Margot Forde Germplasm Center (MFGC) has a large *Trifolium* collection. The center collects and maintains the germplasm of approximately 2000 forage species, including wild and domesticated germplasm, and released cultivars, including intraspecific hybrid cultivars [97]. The Nordic Center for Genetic Resources (NordGen) is a gene bank where *Trifolium* and *Medicago* accessions are also available if used for scientific research purposes [98].

3.2. Phenotypic and Genotypic Characterization and Evaluation of Collected Germplasm, Crossing, and Development of Pre-Breeding Populations

3.2.1. Phenotypic Characterization and Evaluation

Phenotypic and genotypic characterization, evaluation, and screening of genetic resources represent the second step in pre-breeding activities, considering that they are crucial in the selection of material that will be used to introduce new traits into elite cultivars [99]. Humphries et al. [33] acquired 164 perennial *Medicago* accessions and performed a taxonomic classification through preliminary characterization and evaluation in order to identify wild accessions and pre-bred (hybrid) lines (that contain diversity for growth habit, seed size, fall dormancy, and forage yield), but also to introgress these accessions into alfalfa cultivars such that a collection of pre-bred lines could be developed and used in breeding programs all over the world. Solberg et al. [100] compared eight wild populations of red clover (*T. pratense*) with commercial cultivars and landraces according to 13 morphological and phenological traits to emphasize the importance of using wild material in breeding, for the expansion of the gene pool of breeding populations and the facilitation of adaptation to new environments or demands. They also claimed that such research is valuable for practitioners, scientists in gene banks, and researchers/breeders who use gene bank material. Inostroza et al. [44] conducted a study on the phenotypic diversity and productivity of a varied collection of alfalfa (*M. sativa* subsp.), comprising cultivars, landraces, and wild relatives, where they thoroughly analyzed a multitude of morphological and agronomic traits to identify potential materials for inclusion in a selection program aimed at developing drought-tolerant cultivars with exceptional agronomic performance in regions susceptible to drought. Petrauskas et al. [101] evaluated twelve different agromorphological parameters and their biological expression over two consecutive years in wild-type red clover germplasm in 15 individual plants/accessions to determine their phenotypic variability and productivity to identify desirable prototypes in further breeding research. Alfalfa and clover are often grown commercially in densely planted grass swards, and many experimental breeding practices are set up in space plant nurseries, due to seed material limitations and/or individual plant evaluation efficiency [102]. Space plant breeding nursery is a procedure that is generally accepted among breeders and is used in the field to evaluate individual plants and progeny [103]. According to Lutatenekwa et al. [104], characterization indicates the desirable properties of the plant, so the description of the plant on a visual basis (phenotypic characterization of agronomic traits) is very important for the identification of CWRs and landraces to accelerate their selection and utilization, especially in developing countries where molecular markers are expensive.

3.2.2. Genotypic Characterization and Evaluation, Crossing and Development of Pre-Breeding Populations

Plant breeders often use molecular markers for faster analysis of a large number of accessions, identification of desirable traits, and further plant selection. After the phenotypic characterization of agronomic traits (plant height, lodging resistance, green mass yield, grain yield, etc.) is complete, smaller panels of lines, usually around 150 to 300 entries, are created for the determination of phenotype and genotype in detail. Then, marker-trait/QTL (quantitative trait loci) associations can be determined [105]. For the evaluation of plant genetic diversity, molecular markers are the most suitable due to their polymorphism and independence from the environment, such as restriction fragment length polymorphisms (RFLPs), inter-simple sequence repeats (ISSRs), randomly amplified polymorphic DNA (RAPD), simple sequence repeats or microsatellites (SSRs), amplified fragment length polymorphism (AFLP), sequence-tagged sites (STSs), and single nucleotide polymorphisms (SNPs), according to Renganayaki et al. [106] and Mishra et al. [107]. According to Sharma et al. [108], different types of markers like hybridization, PCR, and sequence-based markers are classified based on their throughput as low-, medium-, and high-throughput so they are often referred to as first-generation, second-generation, and third-generation markers, respectively. Understanding the expression of traits, desirable and less desirable, is very

important for breeders focused on the creation of new cultivars. The molecular markers allow for the identification and characterization of genetic variation, where they tag QTLs for the improvement in the target traits and the manipulation of genetic variation [109]. According to Primorac et al. [110], single sequence repeat (SSR) markers have been shown to be a useful tool for assessing genetic diversity in red clover. Qiang et al. [111] used 85 SSR markers to genotype a set of 336 worldwide tetraploid alfalfa genotypes to reveal the genetic diversity and population structure. Thereafter, they found greater genetic diversity in landraces than in CWRs and cultivars, and they also identified two populations corresponding to China and other parts of the world. Bagavathiannan et al. [112], investigated the genetic diversity of 12 CWRs of alfalfa *M. sativa* (Canada), 10 alfalfa cultivars, and *M. falcata* germplasm using eight SSR markers and 14 phenotypic traits. They found a similarity of genetic diversity in wild populations to that of the cultivars, high genetic variation within (99.8%) rather than between different wild populations, and evidence of natural selection in wild populations for winter hardiness, rhizome production, and prostrate growth habit. According to a study in which SNP markers were used to describe the genetic diversity and population structure in 382 samples of red clover (each accession was represented by 10 individual plants), the results showed high genetic similarity between the different cultivars and high genetic diversity of wild populations. The researchers also claimed that it was possible to link the environmental diversity of wild populations with the genetic diversity of cultivated and wild genetic pools [113]. Petrauskas et al. [114] used fingerprinting of inter-single sequence repeat (ISSR) markers for 339 genotypes from 13 populations and 5 cultivars to determine the DNA diversity of red clover, and they discovered high genetic variation (83%) within the populations. McCord et al. [115] constructed a backcross population of 128 progeny by crossing lodging-susceptible and lodging-resistant breeding parents and backcrossing a single F1 plant to the maternal parent (lodging-susceptible) and found significant QTLs for increased forage yield, resistance to lodging, and spring vigor of alfalfa. They further identified the molecular markers associated with these QTLs. The results of the aforementioned studies have provided an opportunity to breeders for further selection and development of pre-breeding populations of clover and alfalfa.

4. Conclusions

The consequences of climate change negatively affect agricultural production and, at the same time, the systematic selection of cultivars with traits that have performed the best over many years has led to a bottleneck in breeding. Frequent and sudden changes in the weather increasingly demand the cultivation of alfalfa and clover in different weather conditions such as drought, heat waves, floods, frost, etc., so the need for widely adapted cultivars has never been greater. By growing legumes such as alfalfa and clover, it is possible to mitigate climate change due to their positive impact on soil and biodiversity, and if researchers go a step further, it is possible to create new widely adapted cultivars by using crop wild relatives and landraces. They have survived diverse and extreme environments over a long period and while their use in breeding programs was once limited, pre-breeding has made it possible to use them more extensively. *M. ruthenica*, *M. sativa*, *M. lupulina*, and *M. arborea* can be cultivated and used as a genetic resource to create tolerant and/or resistant *Medicago* cultivars for low to high salinity areas. Like *Medicago*, *Trifolium* CWRs are also tolerant and/or resistant to various abiotic stresses. CWRs such as *T. purpureum* provide a large amount of biomass in extremely dry areas and can therefore be a genetic resource for creating drought-tolerant cultivars. *T. fragiferum* can be used to create cultivars tolerant and/or resistant to waterlogging, but also to salinity stress. Raunavaara AK0402 can be used to create frost tolerant cultivars as this CWR has survived temperatures down to -12.42 °C. Pre-breeding is a long and extensive process of creating germplasm that can be included in breeding programs, all with the aim of creating new genotypes. Genetic resources can be found in gene banks around the world which have been tasked to preserve, maintain, and provide seed material to breeders for scientific purposes. Not all genetic resources always have the best traits, so it is necessary

to conduct characterization, evaluation, and selection of material that will be used further. Given that the focus of an increasing number of breeders is on the exploitation of wild relatives and landraces in pre-breeding, there is still a lack of available data on individual CRWs, including those found in gene bank databases (basic passport data). Because of this, breeders must invest additional efforts in the characterization and evaluation of a large number of accessions. To shorten the selection process of a large number of genetic resources with desirable traits, it is possible to use molecular markers and quantitative trait loci. Identifying the QTLs and molecular markers for desirable plant traits contributes to plant breeding by determining the number and effect of loci involved in the trait, while eliminating undesirable genotypes before field testing. This review paper gives insight into the problem of genetic bottlenecks in classic breeding and proposes the use of diverse genetic resources and pre-breeding to mitigate the consequences of climate change.

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