Chapter

Reproductive Ecology of Forage Alfalfa (*Medicago sativa* L.): Recent Advances

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Abstract

Plants display an assorted collection of reproductive tactics that eventually play a crucial role in perpetuation of species. Plant reproductive ecology is principally concerned with the adaptive implications of the plant in their vicinity, disparity in qualities allied with pollination, seed dispersal, and seedling establishment. The success in reproduction in most flowering plants depends on ecological interactions with pollinators and seed dispersal agents. Modern tactics in reproductive ecology can integrate proper surveys, advanced pollination studies, interaction between flower and pollinators and clear assessments of population genetic structure, which can provide new opportunities for plant reproductive biology. Alfalfa is an important forage legume and known as "Queen of forages" due to its worldwide adaptability, high yield potential and quality. Alfalfa produces seeds which are primarily used for forage production. It is a gift to livestock industry including dairy, beef, horses, and sheep for grazing, silage, hay etc. Alfalfa is also a medicinal herb with antioxidant, antidiabetic, anti-inflammatory, neuroprotective and cardioprotective properties, utilized for treatment of arthritis, kidney problems. The seeds are exploited in alfalfa sprout industry. The current chapter highlights the reproductive biology of alfalfa from flower development to seed production and its advances.

Keywords: alfalfa, reproductive ecology, forage, seeds

1. Introduction

Reproduction is the critical phase in the life time of any organism, which ensures its perpetuation as a discrete species. The accomplishment of the phenomenon is greatly influenced by two components *viz.*, intrinsic reproductive biology of the organism and the interactive niche in which they survive. The environment in which the plants grow has a major impact as it is decisive in every step of reproduction in plants, due to the fact that the plants are immobile [1]. Therefore, the reproductive success of plant can be constrained by any attributes in the ecosystem where they grow. Alfalfa (*Medicago sativa* L.), commonly called as Lucerne in many other countries is the most important forage crop accounting for the major load of hay produced globally; recognized as the back bone for many forage systems. In Gulf region, alfalfa is widely cultivated and is recognized as the first cultivated forage crop occupying around 30% of the cultivated area [2]. Alfalfa produces nutrient dense foliage with great palatability and digestibility to the cattle. Alfalfa yields better than most forages under less ideal environmental conditions due to its deep root system. Besides, it has long been used medicinally for treatment of arthritis, kidney problems and has antioxidant, antidiabetic, anti-inflammatory, neuroprotective and cardioprotective properties. The factors that impede with the reproductive ecology of alfalfa from reproduction through pollination, seed production and dispersal and the recent advances are highlighted in the current review.

1.1 Plant reproductive biology

Reproductive awareness of any plant is vital for understanding its seed production and dispersal for the continuation of the generation [3]. Plants that do not reproduce vegetatively, depend on seeds to produce offsprings. To be established into a new generation, plants must be pollinated to form fruits, ovules must be fertilized to form viable seeds and the seeds must be dispersed to suitable substrates for germination and growth [4]. Any weak link or break in the chain of events restricts a plant's ability to reproduce and over constant time, contribute to deprivation and impedes the continuation of generation, thereby conservation [5]. Plant reproductive biology may encompass the pollination mechanisms, seed set and seed dispersal between and within populations. Consequently, a comprehensive acquaintance in reproductive knowledge of any plant is mandatory to develop strategies for its conservation [6]. Reproductive biology is a science dealing with the study of the relations between plants and pollinators as well as seed or fruit dispersers. Pollination is an indispensable event in the sexual life of flowering plants, signifying a biotic mutualism between flora and fauna [7]. It is the decisive phase for successive reproduction and suitability of flowering plants for maintenance of seed set and genetic diversity. Pollinators play a key role in reproduction of all flowering plants in the globe, especially agricultural crops depend on pollinators for successful crop production [8]. More than 87% of flowering plants are pollinated by pollinators and insects constitute the major group among pollinators. Plants and pollinators have coevolved to progress mutualistic adaptations, with large ecological changes that dissociate the coincidence in flowering and breeding cycles of pollinators. These coevolutionary relationships are now being severely affected due to global decline in pollinators caused by environmental and anthropogenic disturbances [9]. The human induced interferences such as habitat loss due to fragmentation, deforestation, irrational agricultural practices, extensive use of harmful chemical pesticides, etc. tend to unfasten the advantages of mutual adaptableness of plants and pollinators. The ecological impacts such as pollution and climate change also cause severe threat. Limited pollen or nectar production in plants or ineffective pollinator services due to environmental changes are the chief reasons for the fewness of several important medicinal plants, tree crops etc. [10]. Decline in pollination leads to reduced fruit and seed set, thereby curbing regeneration and limit fitness of plants. Fruit or seed dispersals is vital event for a plant to extend its generation to diverse localities including wild habitats. Thus, the ecoservices by pollinators and dispersal agents errand a crucial part in success of reproduction in a plant.

Observations and experimentation on assorted areas of pollination biology and seed dispersal ecology are a must to comprehend the countless intricacies dominant in plant-pollinator as well as seed dispersal systems [11]. The awareness in this area is a prerequisite for the conservation and management of plant and their associated animal species. Research in reproductive biology of plants will aid to develop new strategies to preserve the genetic potential of the plant species, which are crucial for restoration and reintroduction.

1.2 Plant reproductive ecology

Plant reproductive ecology has arisen as the most adaptable and important fields of plant sciences. It contracts with the reproduction of plants to sustain their generation and the ecological strategies that aid to overcome reproductive catastrophe [12]. For reproductive success of plants owing to its tremendous diversity and ecological niches, substantial amount of disparity is anticipated in the ecological processes [13]. The data base of reproductive biology includes the facts of flowering phenology, floral biology, pollination mechanism, pollen-pistil mechanism, breeding system and natural recruitment.

Lately, the advancement in the particular subject has been stimulated by intensifying interdisciplinarity to address the chief global concerns of warranting food security and biodiversity conservation in the light of several hindrances in plant-pollinator-dispersal agent mutualisms [14]. In this view, the current review describes the reproductive biology of the chief forage plant, alfalfa, from flowering to seed dispersal to enhance the baseline data on its biology and identify deficiencies in its capacity to reproduce and the latest advances to overcome it [15]. The main focus is to synthesize and utilize the database for greater understanding and clarification of the ecological processes governing reproduction.

2. Alfafa: *M. sativa* L.

Alfalfa (*M. sativa* L.) is recognized as the oldest forage grown worldwide, due to its high feeding value and wide adaptability [16]. It is a forage crop with several benefits by strengthening soil structure, contribute nitrogen to soil as well as pest management impacts. Alfalfa is cultivated in more than 80 countries around the globe in an area of 32-million-hectare [17], with a forage yield of 5.75 metric tonnes per hectare per year, hay yield of 8.3 million metric tonnes per year and seed yield of 186–280 kg ha^{-1} annually [18], in addition to the contribution of 83–594 kg N ha^{-1} with the aid of Nitrogen fixing bacteria, *Sinorhizobium meliloti* in the root nodules [19]. Alfalfa is a highly nutritious animal feed and preferred to other forages by the feeding ruminants [20]. The potentiality of alfalfa as a cattle feed arises due to the high content of crude protein with excellent digestibility and its rapid passage through the gastro intestinal track, providing quality proteins for gut microorganisms to resynthesize proteins and vitamins, and stimulate cellulose digestion [21]. The appropriate predicted time of harvest is 10% blooming stage for higher biomass as well as forage yield [22]. As flowering is onset, several physiological functions occur in alfalfa plant. A chief reorganization of photosynthetic possessions from foliage to reproductive parts [23], an increase in lignification of tissues occurs [24], the quality of stem tissues declines and leaf tissues improves [25]. As flowering onsets, a loss in foliage quality up to 45% in relation to feed value is documented. Moreover, a per cent loss in foliage quality leads to 3% loss of cattle daily weight gain [26]. Therefore, a delay in flowering leads to high forage quality for longer period.

2.1 Propagation of alfalfa

Seed is the base for any plant to propagate. Alfalfa is one of the chief forage plants cultivated in dry region through seeds [27]. Seeds are produced in alfalfa for propagation as well as for sprout production for human consumption. Alfalfa can be propagated sexually by seeding alfalfa seeds in a well prepared and well drained seed bed @8–10 kg per acre as pure crop; for mixed strands with grasses, a seed rate of 6–8 kg per acre is required. Asexually alfalfa can be propagated by semi rigid stem cuttings.

2.1.1 Vegetative growing stages of Alfalfa

This interactive diagram demonstrates the different growing stages of alfalfa.

2.1.1.1 Germination and seeding emergence

Seeds should be planted half an inch deeper in the soil in a well-prepared soil, supplied with sufficient nutrients. The alfalfa seeds will absorb water about 24–48 h after planting, provided adequate water is present in the soil. As the seed imbibe water, a radicle root emerges forming the taproot that anchors the seed to the soil surface. The hypocotyl which is the initial seedling develops by pulling the seed coat up through the soil [28]. Germination takes place at optimum temperature range of 65–72°C (**Figure 1**).

2.1.1.2 Seedling growth and establishment

The first visible structure above the ground is cotyledon. A unifoliate leaf emerges as a true leaf, then the trifoliate, which is the second leaf with three leaflets will be produced. As the growth progress the plant produces alternatively arranged trifoliate leaves. Photosynthesis meets all energy requirements by the alfalfa seedling [29]. The first buds develop in the axils of all leaves, at the point of secondary stem emergence.

2.1.1.3 Contractile growth and crown development

The contractile growth occurs in a process where hypocotyl shortens and has a thicker growth due to carbohydrate storage within plant. This stage pulls the

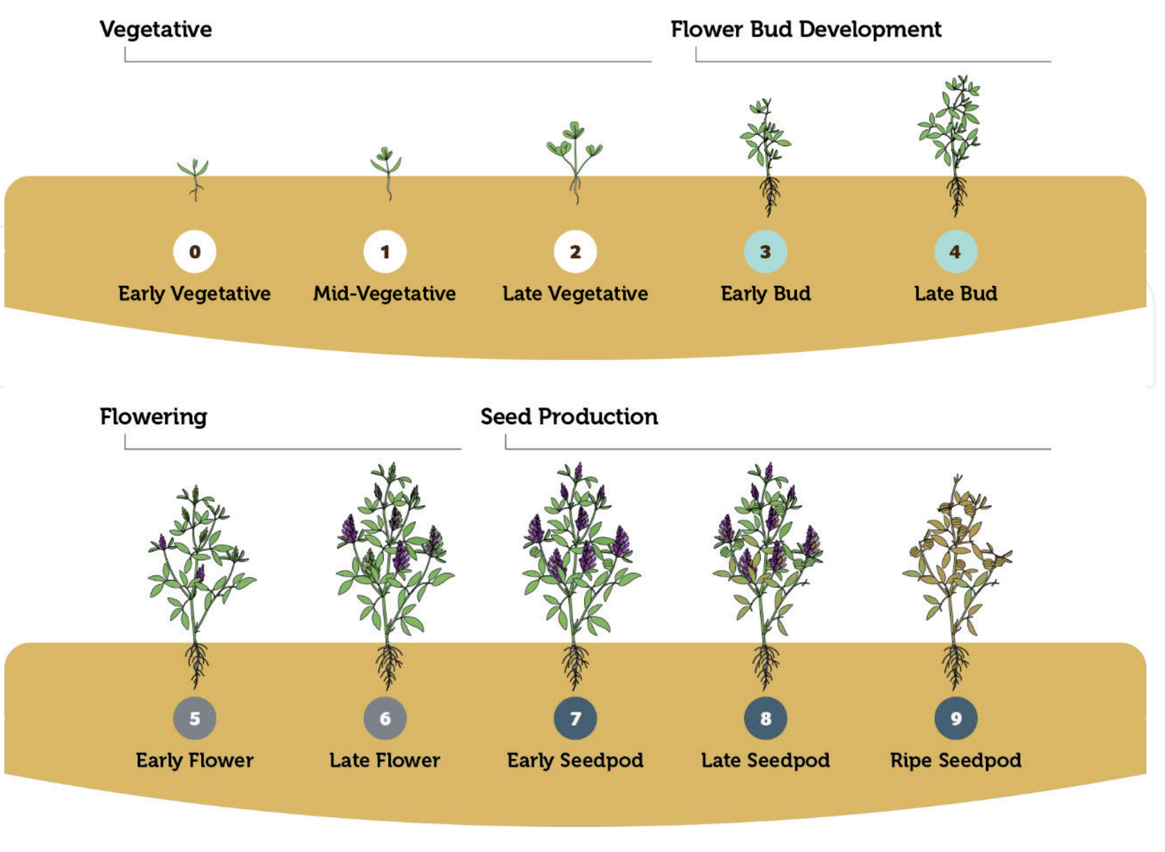

Figure 1.
Growing stages of alfalfa (source: https://nutrien-ekonomics.com/latest-fertilizer-research/alfalfa-development-and-growth-staging)/.

cotyledonary and the unifoliate node underneath and crown develops. At this point of growth, critical crown buds develop. This stage commences from first or second week of emergence and lasts for 2 weeks.

2.1.2 Flowering in alfalfa

The inflorescence of alfalfa is a dense raceme, which is compact around 2 inches long holding up to 50 short-stalked flowers emerging from the leaf axils [30]. The florets commence to open from the base to the tip of the raceme, which takes around a week. A floret may open any hour of the day and remain open for a week if not pollinated, but withers within few hours of pollination (**Figure 2**) [31]. Flowers have five petals, with a larger standard petal upright to curved backward with deep colored veins. The corolla consists of a standard petal, which is the landing platform for pollinators, two smaller wing petals, called lateral petals that project forward and two fused petals called keel. The keel encloses the reproductive parts, two groups of stamen and a style from the green ovary, enclosing anthers and stigma [32]. The color of corolla varies from purple or violet through shades of blue, green, yellow, cream or white [33]. The calyx is short, green with five long pointed lobes with or without fine hair. Each flower has a small pointed bract (**Figure 3**). The sexual organs are non-functional unless released from keel; once released (tripped), fertilization happens if successful pollination occurs. The ovules in the ovary begin to develop, resulting in curly spiral pods twisting in 2 or 3 spirals, the number depends on the number of ovules that progress as complete seed. The number of seeds in a pod depends on degree of pollen compatibility. The pod matures and is equipped for harvest after a month of pollination [34]. A characteristic pod has fine hairs that turns black as matures and release kidney shaped seeds.

2.1.2.1 Tripping

The release of reproductive parts is a phenomenon that is mandatory in alfalfa fertilization and profitable seed production [35, 36]. Tripping is defined as the release of sexual column which includes style, stigma, part of ovary and 10 stamens, by an explosive force as a string under tension is released. Pollinator inserts it proboscis into the flower throat and exerts pressure among the keel petals to access pollen and nectar [37]. When a flower visitor trips the flower, the pistil and stamens hit the pollinators head, where the pollen get deposited and is dislodged by future tripping's of other flowers [38]. Tripping also breaks a membrane on the stigma, which would prevent fertilization of ovule [39]. Natural tripping agents are pollinators such as honey bees and other types of bees and wind [40]. Alfalfa is partially incompatible at fluctuating degrees. If cross-pollinated pollen is not deposited when the flower is tripped, alfalfa may still fertilize by self-pollen (**Figure 4**).

2.1.2.2 Pollination: honey yield, nectar secretion and pollen production

A typical alfalfa plant produces racemes of small pale to dark purple flowers, which require insect visitation for pollination. When a bee visits for pollination, it opens the keel petals and the stamens and pistil move forward, striking the bee with a force. The usual visitors grasp a technique to avoid this mechanism by approaching the flower from the side and inserting their proboscis in between the petals from the base of flower to reach the nectar. Alfalfa secretes a large quantity of nectar, that lures a variety of bee species, of which honey bees produce excellent crop of high-quality honey. The estimated yield of nectar is 416–1933 pounds of nectar per acre [41]. Several terpenoid compounds are identified in alfalfa crops that attracts

Figure 2.
Alfalfa flowers.

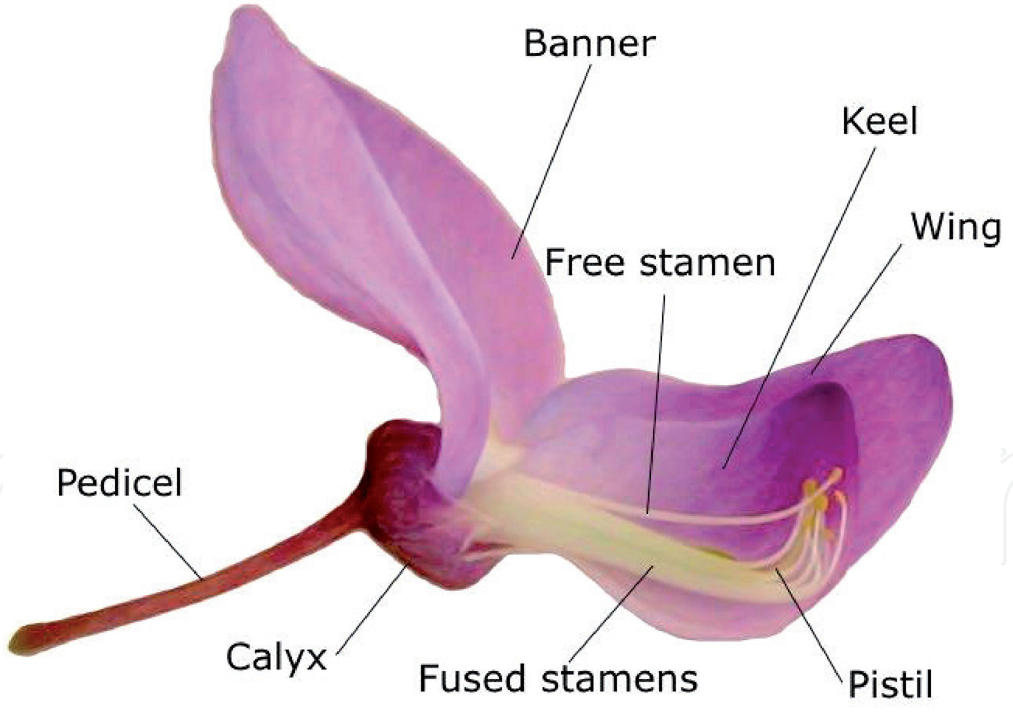

Figure 3.
Inflorescence of alfalfa.

the pollinators towards alfalfa, of which, the aromatic compound ocimene has great role [42, 43]. Alfalfa excretes tremendous quantity of nectar, which lures variety of bees, and from which honey bees produce excellent crops of high-quality honey [41]. Approximately 400–1900 pounds of nectar is secreted by alfalfa [42]. Honey bees are reluctant pollinator of alfalfa as they have to make effort to struck forcefully in the head for the flower to be tripped to enter into forage for nectar. The leaf cutter bee is efficient in pollinating alfalfa than honey bees [44]. High densities

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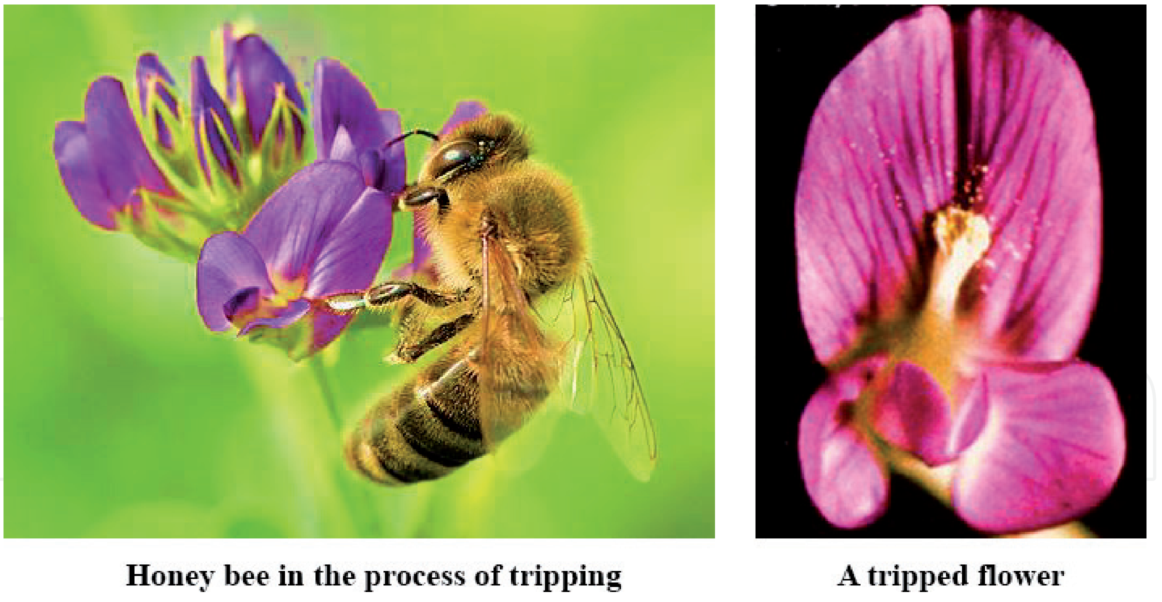

Figure 4.
Tripping.

of bees can lead to adequate pollination to produce desirable honey quantity. A number of 12–15 colonies per acre are recommended for efficient pollination and better honey production. A strong honey bee colony per acre of seed alfalfa could produce 50–100 pounds of honey. Several wild bees also extend their pollination services in alfalfa [45]. Breeding alfalfa cultivars more friendly to honey bees with traits such as plants with stamen protruding beyond keel, plants that could be more easily tripped, plants with high nectar production etc. will aid in greater pollination services, thereby more seed set [46]. Bee colonies in alfalfa crop, yields honey potentially as they are the chief alfalfa pollinators. If the crop is cut for hay before flowering, the bee keeper will get very little quantity of honey [47]. If the crop is for seed production, a better yield of honey can be harvested, which depends on the plant density, competition from other pollinators and other environmental as well as agronomic factors. Alfalfa is a poor source of pollen for honey bees, but relished by other pollinators such as *Bombus*, *Megachile*, *Halictus* and *Nomia* [48].

2.1.2.3 Seed set

A miniature portion of the total ovules develop into seed. A flower should be visited at least once by a pollinator, to get tripped before fertilization. Once tripped, the racemes hold the developing seed pods on the lower part, few open florets in the middle and unopen buds at the top [49]. The cross-pollinated pods produce comparatively more seeds than self-pollinated plants. Moreover, the plants regenerated from self-pollinated plants are less competitive with low survival rates. It is estimated that 2 billion flowers per acre of alfalfa were produced, of which 200 million flowers have the ability to set pods. In a probability of 5 seeds per pod, 2,200,000 seeds per pound, a potential seed production of 50,000 pounds of seed per acre is estimated [50].

2.1.2.4 Seed dispersal

Alfalfa seeds are stood in coiled leguminous pod and it is nonshattered. The local mode of dispersal is natural and non-mechanized. Wind dispersal of seeds is very rare as the seeds are very dense and smooth. Seed dispersal by animals is a possible mode, in situations where animals forage on them [51].

2.1.2.5 Seed quality

Seed quality is the critical aspect in agriculture for offspring generation as well as their conservation, which is measured by germination percentage. The factors influencing seed quality includes proper irrigation and nutrient supply, climatic conditions during seed formation, maturation, harvesting, and drying techniques. Seeding in suitable field condition at proper timing, smearing good crop management aspects, adopting suitable harvesting and drying techniques, minimizing mechanical injuries and ensuring minimum deterioration in storage will favor in obtaining alfalfa seeds of prior quality [52].

2.2 Taxonomy and genetics

Medicago sativa L. belongs to the order Fabales, family Fabaceae, tribe Trifolieae, genus *Medicago*. The genus *Medicago*, is wide, comprises more than 50 species; mostly annuals and few species are perennials [53]. *M. sativa* is a perennial herbaceous legume, that is a tetraploid (2n = 4x = 16) [54]. The allogamy and autotetraploid nature of this species contribute to genetic variations within the population. The commercially cultivated species for forage is *M. sativa* belongs to a *M. sativa* complex, a group of closely related sub species sharing similar karyotypes, the collection of chromosomes and interfertile, capable of interbreeding. The diverse subspecies in the complex comprises subsp. *glutinosa*, subsp. *coerulea* etc. [55]. The most commonly cultivated alfalfa in the globe is *M. sativa* and *M. falcata* is cultivated to some extend around the globe. *M. sativa* is a tetraploid, characterized by purple flowers and coiled pods, whereas *M. falcata* occurs both as tetraploid and diploid and characterized as yellow flowers and sickle shaped. Two other closely related species *M. prostrata* and *M. glomerata* is utilized in natural hybridization with alfalfa [56]. All other members of *M. sativa* species readily cross pollinate with the cultivated species. Several alfalfa species exhibit genetic self-incompatibility or self-sterility and will not successfully self-pollinate [57]. Alfalfa is severely affected by inbreeding depression i.e. reduced forage and seed yield due to self-fertilization attributed to accumulation of recessive alleles due to self-pollination or pollination among close relatives. Alfalfa plays a great role in any genetic engineering context in their improvement as it is an allogamous tetraploid, highly sensitive to inbreeding depression, inability to survive and perpetuate its genetic material [58], highly variable among cultivars and most are synthetic cultivars produced by recurrent phenotypic selection [59]. Therefore, advancement in alfalfa readily lies in other species or sub species of economic importance.

2.3 Ecology of alfalfa

Alfalfa is well adapted to most environments as demonstrated by its wide global distribution. The perennial growth habit of alfalfa, ability to survive extreme temperature, nitrogen fixing symbiosis through their roots and their hard seed coat facilitate their survival in extended ecological regions [60]. High summer temperature, drought, competition from other grasses, water logging and root and crown diseases leads to stand loss [61].

Hard seed: Hard seed is a post-harvest dormancy attributed by alfalfa seeds. A hard water impervious seed coat temporarily prevents water uptake, thereby delays germination. Majority of alfalfa seeds imbibe water and germinate readily and not exhibiting true physiological dormancy [62]. **Auto toxicity:** Alfalfa plants and plant residues produce compounds that provoke an autotoxic reaction that hinder seed germination [63].

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2.4 Reproductive ecology of alfalfa

Reproductive success in alfalfa occurs through interaction between development and organization of flower, pollination, fertilization, seed formation, dispersal and other ecological factors. Flowering onset is an intricate progressive evolution that could be activated by various pathways [64]. Diverse ecological and plant internal factors like day length, temperature, light quality and age of plant mediate this transition [65–67]; temperature and photoperiod are important signals in alfalfa flower induction [68, 69]. Alfalfa is a long day plant, requiring 12–14 h of minimal brightness to induce flowering [70]. Alfalfa is solely an insect pollinated crop, which is pollinated only by a small group of insects, especially bees. Each alfalfa bloom is pollinated by a single insect in a single time. The pollinator species, the genotype of alfalfa and the nectar content can affect the pollination process of alfalfa. The predominant pollinators of alfalfa are honey bees (*Apis mellifera*), leaf cutter bees *(Megachile rotunda*) and alkali bees (*Noma melanderi*). Alfalfa seed requires 4–6 weeks to develop into a viable seed [71]. The environmental factors such as rainfall and low temperature during maturing process will decrease the viability of the seeds, ultimately producing seeds of poor quality. The seedling vigor will be reduced and the germination quality will be minimized due to fungal infection or premature seed sprouting [72]. Genetic differences between varieties also affect seed maturity.

3. Advances in improving reproductive ecology of alfalfa

Alfalfa, well known for its extensive ecological adaptability, with development of modern breeding and genetic engineering techniques has advanced in reproductive ecological aspects. Genetic engineering approaches in alfalfa breeding is utilized to manipulate the expression of genes involved in metabolic pathways to improve the reproductive traits, pollination and seed quality traits.

3.1 Breeding and seed production

Any alfalfa cultivar has around 10–200 parents, crossed in isolation to produce breeder seeds. A group of plants distinct for a specific trait, preserved through 2–3 generations from other alfalfa cultivars and cultivars produced by this kind of breeding is called synthetic cultivars. Most commercial alfalfa cultivars are synthetics produced from advanced progenies of clones of superior traits [73]. The out-crossing nature of the alfalfa plant and its polyploid genome create complexity in genetic improvement for higher forage and seed production. The prevalence of severe inbreeding depression prevents researchers from capturing heterosis in alfalfa cultivars through hybrid development. Consequently, it has led to a modification in breeding strategy for higher yield, by intercrossing selected parents to produce synthetic cultivars [74]. This strategy is currently more feasible than the development of a hybrid cultivar. The intercrossing approach between plants with a broad genetic base increase heterozygosity, which increases the intra-locus interaction, and ultimately yield.

3.1.1 Alfalfa, a potential plant for genetic research

The genus *Medicago* is scattered worldwide and comprises 83 species [75]. The cultivated alfalfa for fodder purpose is autotetraploid, cross-pollinated and seed propagated [76]. A rich source of natural variation and genetic resources is held by three species *M. sativa*, *M. falcata* and *M. media*, with breeding potential for development of new cultivars. Genetic diversity studies of any species are indispensable for successful breeding into new cultivars. Variations of a population, genetically and phenotypically give insights in evolutionary history [77]. A study with 25 accessions of the three *Medicago* species collected in Leh Region, Kashmir, India, analyzed using simple sequence Repeats (ISSRs) and random amplified polymorphic DNA revealed a high genetic variation. A genetic diversity of 30.23% within populations with a mean coefficient of differentiation (Gst) was noticed. The overall value of mean estimated number of gene flow (Nm = 8.0682) revealed large gene exchanges among populations. Analysis of molecular variance (AMOVA) states the genetic diversity being 49% among populations and 51% between populations. The distinct genetic variation in *Medicago* species is an ideal plant for genetic research for breeding for improved forage varieties.

3.1.2 Breeding to overcome inbreeding expression

Several breeding programs are structured to avoid or overlook the negative impacts of inbreeding expression in alfalfa [72]. Inbreeding expression occurs due to continuous selfing. The vigor of natural tetraploid alfalfa is reduced by inbreeding depression, but maximizing heterozygosity offers a better solution [92]. The intra-locus interaction and additive variation maximizes heterogeneity in alfalfa, to enhance its performance [78]. The forage yield of alfalfa is experienced due to increased frequency of favorable alleles and utilization of non-genetic effects in a study for long years (1898–1985). The study also revealed the enhanced genetic load in recent alfalfa cultivars due to several crossings, which hinders the lethal alleles at their heterozygous loci, providing better germplasm source of alfalfa [79].

3.2 Advances in floral biology

3.2.1 Effect of anatomical structure of the alfalfa flower in tripping mechanism

Alfalfa possess a highly unique papilionaceous flower with an exclusive tripping mechanism, which permits only certain insects to enter in to effect tripping [94]. *M. sativa* was studied at the blossom stage to correlate the part of flower tripped by pollinators in relation to the blossom characters. The comprehensive study on the anatomical structure of flowers of 11 selections displayed two chief services involved in tripping mechanisms *viz.*, a cohesive force between the two keel petals and the pressure exerted by the sexual column from cells under tension at the point of fused filaments and the keel. The initial force was resulted due to the interlocking of the projections on the pressed surfaces of keel petals, which is adequate to prevent spontaneous tripping of alfalfa flowers. The firmness of the preventive force and the relative pressure exerted by the sexual column against the appressed keel petals differs in flowers from various selections which is attributed to differences in anatomical structure and development of blossoms. The results revealed that the preventive force of the adhered keel petals is closely related to the proportion of insect tripped flowers than the force with which the sexual column is released by tripping [80].

3.2.2 Mutants of varied inflorescence patterns in alfalfa

Alfalfa is a typical leguminous plant with a typical raceme inflorescence. The multi-inbreeding process in this cross-pollinated species, develop several mutant forms with long, panicle-like racemes, with fertile and sterile flowers, complex branched racemes, fascinated racemes etc. The transitional form of some mutants is isolated by means of pair hybridization and new mutant forms were developed. New mutants with diverse inflorescence pattern provide room for more pollinator's activity leading to improved seed set. *Medicago truncculata* shared highly conserved nucleotide sequence and exhibit perfect synchrony between genomes. *M. truncculata* mutant *mtpim* has a complicated inflorescence resembling panicle, controlled by spatiotemporal expression of MtTFL1, MtFULC, MtAP1 and SG1 through reciprocal repression. Some of them resemble *M. sativa* phenotypes. The mutant developed by retrotransposon insertion mutagenesis sg1l-1 has a cauliflower type phenotype resembling a mutant of alfalfa [81]. The data generated on genes regulating inflorescence developed in *Medicago* species helps to understand the phenomenon of inflorescence mutations in alfalfa, which is helpful for modification in inflorescence structure for enhanced pollination.

3.2.3 Ovule sterility and seed set in alfalfa

The seed potential of alfalfa is very low; an estimated seed to ovule ratio is about 0.08 [82]. This deficit is due to the low number of seeds produced per pod; of 10 ovules present in a floret, only an average of 5 develops into seed. Eliminating at least few causes that limit seed potential will be beneficial in improving seed yield of alfalfa. An ovule sterility trait allied with limiting integument formation controlled by a single recessive gene has been developed in alfalfa. In alfalfa cultivar, Blazer XL, an ovule sterility trait B17, with 81% ovules displaying heavy callose deposition at the time of anthesis with low female fertility was reported [83]. These plants were female sterile when hand crossed with unrelated male fertile plant. In high percentage of ovule sterile plants, under sized pistils develop at anthesis, that will not emerge from staminal column. A mapping study of chromosome region revealed and explains a major share of variation for ovule sterility and the cytological analyses showed that no embryo sac develops in sterile ovules and the callose deposition begins after meiotic division [84], affecting the integumentary tapetum and nuclear cell walls, which usually expands to fill the space occupied by the embryo sac [85]. The pistil growth often ceases at bud stage and a short-aborted pistil is found within the staminal column at anthesis, in a plant with 100% sterile ovules [86]. Nine populations analyzed for ovule sterility showed 4–26% sterile ovules with significant negative correlation between percentage of sterile ovules and seeds per pod in most populations [87]. A quick stain clearing technique based on callose fluorescence is effective to trace ovule sterility in breeding programs. In addition, checking ovule fertility in parental genotypes aid breeders to develop good seed yielding cultivars.

3.2.4 Effect of floral nectaries and flower aroma of alfalfa

Alfalfa secrets nectar at a uniform daily rate for 4–5 days after flower opening. The volume of the nectar produced differs per floret, which are found to be heritable. Two plants each from a high, intermediate and low nectar producing alfalfa cultivars were subjected to light and scanning electron microscopy, and the images designated nectary located on the staminal column of the receptacle [88]. It comprises of several cell layers subtended by vascular bundles containing both xylem and phloem, but not extend into nectariferous tissue. The epidermal layer comprises permanently open stomata, which functions in nectar secretion. The number of stomata per nectary among the six clones ranged from 24.7 ± 1.9 to 6.8 ± 0.5. The nectar-reservoir diameters ranged from 1.07 ± 0.09 mm to 0.70 ± 0.01 mm. The cultivars with the largest nectar reservoir witnessed high number of stomata [89].

Flower aroma of alfalfa has a specific role in pollinator attraction. Seven clonal lines of alfalfa with difference in flower aroma were consecutively recorded for honey bee visits at different locality and volatiles were also extracted from the flowers. A gas chromatographic study suggests a difference in volatile components [90]. Therefore, the compounds present in the volatiles of nectar has a major role in pollinator attraction for enhanced seed set.

3.2.5 Features of alfalfa flower that effect seed production

The characters and properties of alfalfa flowers investigated to determine their role in seed setting revealed that the affluence to trip the flower is allied with the age of floret. The age of floret does not have an effect in self-fertilization, but declines for 3 days in cross fertilization. Pollen from untripped florets showed decline in germination, but remained viable when stored in honey bee colony. The honey bees that collect pollen clean themselves with pollen in 2 days. There was no steadfast increase in seed production due to repeated visits of honey bees. Dusting of foreign pollen before tripping has not increased cross fertilization, but 50% increase in self-fertilization. Ovules of florets with exposed stigmas remained functional up to 6 h and then declined rapidly for next 2 days. Self and cross-fertilized seeds were amalgamated in the pods but self-fertilized seeds did not occur beyond the fourth position from the proximal end. Studies suggested that the honey bee pollination can be improved by providing an alfalfa flower with an exposed stigma [91].

3.3 Influence of pollinators in alfalfa pollination and potential for gene flow

Diverse pollinators have diverse roles in pollinating specific crops. Though managed pollinators, are utilized for pollination of alfalfa for seed production, several wild pollinators also visit them [92–95]. Diverse pollinators display assorted efficiency in depositing and detaching pollen from individual flowers [96, 97]. The rate of tripping and the quantity of pollen deposited varies between pollinator species visiting the racemes [98], which depends on whether the pollinator forages for pollen or for nectar [99] and influence on seed set. Apart from influencing pollination, the pollinators differentially affect gene flow [100]. The bumble bees carry pollen for short distances, but not the hawkmoths. In addition to pollinators, various features of field that grow alfalfa also plays a major role. Increased density of plants has displayed reduced gene flow as pollinators respond to locally available floral resources and shorten the flight distance [101–103]. The load of pollen carried by the pollinators from donor to recipient is expected to turn over quickly as pollinator visits greater number of plants per unit distance traveled, which declines the gene flow.

Though transgenic alfalfa plants are developed, the impact of pollinators on gene flow is yet to be studied as various wild insect pollinators contribute to pollination and movement of genes [104]. A model to predict gene flow between transgenic and conventional field [105], predicted the number of visits by the pollinator in a foraging session, estimates the extent to which the transgenic pollen is diluted by conventional pollen. Essentially, the amount of transgenic pollen on conventional field flower is inversely proportional to the total amount of pollen delivered by each bee during a foraging session in conventional alfalfa. Accordingly, the greater number of flowers the specific pollinator visits, the minimal amount of transmission of transgenic pollen and its fruit set [106]. A study to investigate the involvement of managed honey bees (*A. mellifera*) and leafcutter bees (*Megachile rotundata*), three bumble bee species (*Bombus impatiens*, *Bombus griseocollis*, *Bombus auricomus*) and two solitary bee species to pollination (*Halictus rubicundus* and *Andrena asteris*) and their contribution in gene flow revealed the potential role of two wild solitary bees and a wild bumble bee in tripping, while the managed pollinators, *A. mellifera* and *M. rotundata* recorded the least. Honey bees, recorded the best potential in gene flow and reduced transgene transmission in relation to other pollinators. The denser plant stand of alfalfa does not show any impact in gene flow and reduced transgene transmission, while the three bumble bee species portrayed an increased gene flow and reduced transgene escape in high density alfalfa plantations [107].

3.3.1 Gene flow in commercial seed production

Alfalfa being an outcrossing and insect pollinated crop, the potential for gene flow has been widely recognized. Gene flow is the exchange of genes from one population to another. It is a natural mechanism that changes the genetic frequency of population over time to enrich the wealth of biological diversity. The formation of cross-pollinated genes and their establishment mediates occurrence of real gene flow [108]. Though pollinator species carry pollen to long distances, true gene flow can occur if the pollen can produce viable seeds and thus offspring. Pollen mediated gene flow is greatly dependent on physical isolation distance between two populations, the degree of synchronous flowering, availability of pollinator gene frequency etc. [109]. Maintaining adequate isolation distances between any alfalfa and seed production trials help to minimize the bee-mediated gene movements. A minimum of 50 m between the alfalfa crops for certified seed production and 200 m for plots exceeding 5 acres and 300 m for plots of 5 acres or less for foundation seed production is recommended [110, 111]. In seed alfalfa production for commercial purpose, bees are purposely stocked in the field. Gene flow, rate of outcrossing, pollinator type and behavior are very important for proper management of commercial alfalfa seed production. Advanced genetic technologies offer better tools to understand the dynamics of pollinator-mediated gene flow. A study to measure the gene flow using honey bee pollination under commercial alfalfa seed production reported a significant decrease in gene flow with increase in isolation distance. A 900 ft. colonies of honey-bee (*A. mellifera*) mediated gene flow was 1.49% and it decreased linearly to 0.20% near 5000 ft. Gene flow continue to decline with increase in isolation distance [112]. The same was reported for alfalfa leaf cutter bee (*M. rotundata*) [113]. Another research with Roundup Ready Trait as a marker gene flow examined the movement of the gene *epsps* from the Roundup Ready plots to conventional alfalfa plots isolated by 152, 274, 610 and 825 m distance. With leaf cutter bee as the pollinator, the pooled data from 2000 to 2002 revealed that the upper bound (99.9% confidence) of gene flow at 274 m was 0.3%, at 152 m was 1.7% and gene flow was not detected at 610 and 825 m at years 2000 and 2002. The results confirmed the role of isolation distance as an effective means to maintain purity of both Roundup Ready and conventional alfalfa plots [114].

3.3.2 Gene flow in forage alfalfa production

Pollen flow or dispersal is a form where genes can move between plants. However, the total pollen dispersed will not cause gene flow, which is the successful transfer of genetic material. As pollen-mediated gene flow occurs between sexually compatible plants and deposited on stigma of a plant, it fertilizes the ovule, resulting in production of viable seed. Flowering in alfalfa reduces the forage and nutritive value and it has significant consideration in gene flow of alfalfa. Alfalfa managed for forage production is cut several times a year from two to ten, depending upon the climatic factors of the region and the stage of alfalfa development which is early to 10% blooming stage. Periodical harvesting removes the entire plant canopy with flowers or seeds. For high quality forage, first cutting occurs in mid to late bud stage [115]. The cutting interval is 28–35 days, an interval well adequate to initiate full bloom and matured seed. Harvest of alfalfa as dairy feed eliminated the entire plant canopy terminating bloom and seed formation. Regrowth of canopy reinitiated from vegetative crown buds and elongation of lower stem axillary buds. Therefore, alfalfa managed for forage production will have minimal contribution to gene flow, as flower blooming happens in a very less percent and thus pollen transfer. Gene flow from Roundup trait alfalfa to conventional alfalfa for forage production is predicted to be far less as compared to seed production alfalfa [116]. Accordingly, the gene flow from genetic engineered alfalfa grown for forage to conventional forage field is lower than alfalfa grown for seed.

4. Climate change

Alfalfa, extends its timely service in climate regulation by drawing carbon from atmosphere and dumping deep in soil. But climate change effected by global warming and enhanced nitrogen deposition directly or indirectly affect the reproductive characters of alfalfa. The shift in phenology and foraging crop distribution of plants and pollinators leads to temporal decoupling and spatial mismatch between them [117]. Climate change may affect the floral traits such as floral display, corolla structure, formation and position of ovule and stamen, subsequently affecting the quality and quantity of forage of pollinators, ultimately the reproductive success in plants. Insect pollinators are valuable resources but are limited. Although around 20,000–30,000 bee species are present worldwide, only 10–11 species are managed [118], and *A. mellifera* is far being the most dominant species managed globally. Biotic stress convoyed with climate change causes severe decline in pollinator population. The genus *Apis* is in risk due to climate change and the mismatch in foraging plants and pollinator regimes, thus there is a want for alternate pollinators. Well-known pollinator, *A. mellifera* is being replaced by leaf cutter bee (*M. rotundata*) and alkali bees (*Nomia melanderi*) in alfalfa ecosystem [119].

5. Future scientific challenges and perspectives

Alfalfa cultivars are usually synthetic populations that originated by a heterogenous blend of heterozygous genotypes, that thwarts the genomic results in breeding process. The application and implementation of genomic techniques for genetically improved alfalfa may be quite promising and challenging, as most matters could be solved at technical and commercial level. Even though, plant genetic engineering encounters the chances for improvement of alfalfa cultivars to some extent, but there are still challenges [120]. Gene transfer techniques involve appropriate use of promoters, transit peptides, choice of selectable or reporter markers etc. The promotors specific to alfalfa are inadequate and fully characterized expressing genes in high level. Consequently, a convention of constitutive, specific promoters effective to alfalfa are mandatory for augmented expression of transgenic research. Therefore, a set of constitutive, tissue or temporal-specific promoters effective in alfalfa is obligatory for the optimized expression of transgenic research [121]. As alfalfa is tetraploid, the transgene integration and gene stacking techniques utilized for other crops are not suitable for alfalfa. Innovative breeding approaches would be needed to adopt and address these challenges in alfalfa.

6. Conclusion

Reproduction is a crucial phase in the life period of any organisms, which ensures their endurance as a distinct species and governing the adaptability of plants to multivariate ecosystem. The precise design of resource distribution for reproduction depends on the reproductive parts and their union, factors influencing the fertilization, genetic variation and resource availability. Plants choose diverse reproductive modes based on the resource and pollination deficiency. Self-pollination and clonal reproduction are conducive to reproductive assurance in stands with pollinator scarcity, while sexual reproduction and cross-pollination improves seed quality and genetic diversity of progenies, which would benefit population survival. Consequently, the amalgamations of assorted reproductive modes and generalized pollinator networks may be good choices to adapt successful reproduction. Awareness on the key processes can be useful in addressing the current challenges, that impact the ecosystem directly or indirectly. The evolution of floral traits might be obsessed by selective pressure of local pollinators, exotic pollinators, nectar robbers and abiotic environments such as temperature, precipitation and ultraviolet radiation on several traits related to flower. The applications of new technologies and methods such as breeding to overcome inbreeding depression could advance the awareness on alfalfa reproductive ecology. Advanced floral mechanisms such as altered anatomical structure of the alfalfa flower in tripping mechanism, mutants of varied inflorescence patterns, ovule sterility and seed set, floral nectaries, floral aroma and flower features of alfalfa has major influence on altering its reproductive ecology. Outstandingly, the progress in high-throughput sequencing of alfalfa cultivars genetically, has brought an outlet for the various genetic shortcomings in alfalfa mass production accomplished by breeding and biotechnological means. The fundamental mechanisms on diverse topics of plant reproductive ecology could be discovered more effortlessly, especially in relation to floral traits and pollinators for better alfalfa production in future researches.

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