CHAPTER 4

Quantitative genetics applied to improvement of alfalfa

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Introduction

One barrier that prevents alfala expansion in Brazil is the lack of cultivars adapted to tropical conditions. In order to understand the real dimension of this problem, it would be sufficient to mention that despite the great demand for new releases from the Brazilian market, the only currently available cultivar is Crioula, a domestic variety with good adaptability and good stability throughout the country (Ferreira et al., 2004).

The development of new cultivars will enable the use of alfalfa in different regions of Brazil, with the consequent increase not only in the alfalfa acreage but also in the availability of high quality feed for intensive milk production systems (Botrel et al., 2001).

The expansion of an introduced exotic species depends on its adaptation to the conditions of the new environment. In this context, cultivars from temperate regions, as is the case of alfalfa, normally have problems adapting to the tropics, since the selective pressure exerted during the breeding process has not included adaptation to a tropical environment (Ferreira; Pereira, 1999).

Breeding methods are a useful tool for developing adapted cultivars starting from exotic materials, assuming there is enough genetic variability in the germplasm of the species. By recombining only selected genotypes, the frequency of favorable alleles in the population is increased and thus it is more likely to achieve effective selection gains in the breeding of the species (Allard, 1971; Rumbaugh et al., 1988; Basigalup, 2007).

In this chapter, the implications of allogamy and autotetraploid inheritance on alfalfa genetic improvement will be addressed. In doing so, some quantitative genetic aspects – such as gamete formation and gamete segregation, gain from selection, response to selection, genetic variance components, heritability, inbreeding, heterosis and inbreeding depression – will be briefly discussed.

Reproductive system

Cultivated alfalfa is a perennial autotetraploid (2n = 4x = 32) species, with perfect flowers and mainly allogamous fertilization. It has self-sterility and self-incompatibility mechanisms that prevent selfing.

Natural pollination is carried out mainly by bees. Because of the pollination control mechanisms, pollinators must visit different flowers, and thus forcing cross-pollination.

Alfalfa is a polymorphic species, with diploid and tetraploid forms. Its basic chromosome number is eight. Since cultivated alfalfa is autotetraploid,

inheritance of traits is complex and has profound consequences on its genetic behavior and on the breeding methods to be used for its improvement.

Quantitative genetics of alfalfa

Biometric methods used by breeders to improve traits related to production and to quality of alfalfa are based on the breeding system and the genetic structure of the species.

Segregation and gamete formation

In discussing this section, two assumptions are made: a) chromosome segregation in alfalfa is at random; and b) the existence of double reduction and preferential pairing, as well as the non-disjunction of chromosomes, is ignored.

Of these factors, only preferential pairing can bring significant deviations to what is expected from theory. However, other phenomena such as preferential pollination of flowers by insects, differential pollen-tube growth rate, incompatibility, sterility and abortion of fertilized ovules, can also cause deviations from the expected results (Busbice et al., 1972).

In a single locus with four alleles (tetraploid), five possible genotypes can be observed: the first, with four dominant alleles (AAAA), is called "quadruplex"; the second, having three dominant alleles (AAAa), is called "triplex"; the third, possessing two dominant alleles (AAaa), is called "duplex"; the fourth, having only one dominant allele (Aaaa), is called "simplex"; and the fifth, with no dominant alleles (aaaa), is called "nulliplex" (Blakeslee et al., 1923).

In the case of complete dominance, the dominant trait can be observed when there is at least one dominant allele; thus, the recessive trait should be observed only under the nulliplex condition. However, in most cases the dominant phenotype in alfalfa is expressed only when two or more dominant alleles are present (Whittington; Bubrage, 1963; Pedersen; Barnes, 1965).

Tetraploid individuals produce diploid gametes. Based on the alleles present at a single locus, these gametes may have different structure and different probabilities of segregation, as shown in Table 1. For example, AAAA individuals only produce AA gametes, with probability equal to 1, unlike AAaa genotypes which can produce three types of gametes: AA with probability 1/6, Aa with probability 4/6 and aa with probability 1/6.

Based on these probabilities, it is possible to obtain the number of individuals to assess from a cross, in order to detect specific genotypes. For

Table 1. Probability of gamete segregation at a single locus by tetraploid individuals with different genotypic constitution (chromosome segregation).

Genotype of the individual	Diploid gametes		
	AA	Aa	aa
AAAA	1	0	0
AAAa	1/2	1/2	0
AAaa	1/6	4/6	1/6
Aaaa	0	1/2	1/2
aaaa	0	0	1

instance, families produced by self-fertilization (selfing) of a duplex individual (AAaa) will produce nulliplex individuals with probability of 1/36 (1/6 x 1/6). If instead of selfing, a test cross is carried out, the probability of obtaining the nulliplex individual is 1/6 (probability of obtaining the aa gamete produced by the duplex individual). Therefore, to identify a nulliplex individual with a 95% level of confidence, 107 self-fertilized offspring should be analyzed, while with the test cross it would be necessary to assess only 17 progeny to achieve the same probability.

The analysis of the evolution of the genetic structure of a tetraploid population under different mating systems is essencial to understand the breeding behavior of a tetraploid species, especially those aspects referred to genetic equilibrium.

Genetic equilibrium in an autotetraploid population can be estimated by comparing the gametic frequencies produced by such population over two or more generations. In this context, when the gametic ratio of the population does not change from one generation to another, the population is in genetic equilibrium. As an example, it can be considered the case in which the initial population has the following structure: 0.13 AAAA: 0.16 AAAa: 0.06 AAaa: 0.08 Aaaa: 0.57 aaaa (Table 2). In doing the calculations, random mating will be assumed. The genotypic ratio of the offspring is given by the square of parent gametic ratio, that is: genotypic ratio of offspring = [gametic ratio of parents]^2.

Thus, probability of each gamete in the parental population (P_0) is: $P(AA)_0 = 0.22$; $P(Aa)_0 = 0.16$ and $P(aa)_0 = 0.62$.

Table 2. Frequency of parental genotypes and frequency of diploid gametes produced by those parental genotypes in a population of autotetraploid plants.

Genotype of the parents	Frequency	Diploid gametes of the parents		
		AA	Aa	aa
AAAA	0.13	0.13	0.00	0.00
AAAa	0.16	0.08	0.08	0.00
AAaa	0.06	0.01	0.04	0.01
Aaaa	0.08	0.00	0.04	0.04
aaaa	0.57	0.00	0.00	0.57
Result	1.00	0.22	0.16	0.62

Therefore, the genotypic ratio in the offspring will be = $(0.22\,AA + 0.16\,Aa + 0.62\,aa)^2$, resulting in this proportion of individuals 0.0484 AAAA: 0.0704 AAAa: 0.2984 AAaa: 0.1984 Aaaa: 0.3844 aaaa.

The next generation of the previous population will have the gametic proportion (P_1) that is shown in Table 3.

That is:

$P(AA)_1 = 0.1333$; $P(Aa)_1 = 0.3333$ and $P(aa)_1 = 0.5333$.

As a consequence, since $P(AA)_0 \neq P(AA)_1$; $P(Aa)_0 \neq P(Aa)_1$ and $P(aa)_0 \neq P(aa)_1$.

Table 3. Frequency of offspring genotypes and frequency of diploid gametes produced by this offspring in the population of autotetraploid plants derived from the parental population described in Table 2.

Offspring genotype	Frequency	Offspring diploid gametes		
		AA	Aa	aa
AAAA	0.0484	0.0484	0.0000	0.0000
AAAa	0.0704	0.0352	0.0352	0.0000
AAaa	0.2984	0.0497	0.1989	0.0497
Aaaa	0.1984	0.0000	0.0992	0.0992
aaaa	0.3844	0.0000	0.0000	0.3844
Total	1.0000	0.1333	0.3333	0.5333

It is concluded that the population in this example was not in equilibrium and that genetic equilibrium was not achieved after one generation of random mating. While the latter is a distinctive condition of autotetraploid populations, one generation of random mating is sufficient to reach equilibrium in diploid populations.

For an autotetraploid population at Hardy-Weinberg equilibrium and under random mating, assuming the gametic frequencies of A and a are represented by $f(A) = p$ and $f(a) = q$, the frequency of the five possible genotypes is given by the equation $(p + q)^4$. For the precious example, in which $f(A) = p = 0.3$ and $f(a) = q = 0.7$, the resulting genotypic frequencies are shown in Table 4.

All of the above has practical implications in the alfalfa breeding. As an example, the assessment of the offspring derived from the cross of two tetrallelic individuals can be considered, as shown in Table 5.

Table 4. Genotypic frequency in a population of autotetraploid plants at Hardy-Weinberg equilibrium.

Genotypes	Frequency	Genotypic frequency in equilibrium
AAAA	p^4	0.0081
AAAa	$4p^3q$	0.0756
AAaa	$6p^2q^2$	0.2646
Aaaa	$4pq^3$	0.4116
Aaaa	q^4	0.2401

Table 5. Possible gametes produced by two different tetrallelic alfalfa parental individuals (A_1-A_4 and A_5-A_8).

Individuals	$A_1 A_2 A_3 A_4$	x	$A_5 A_6 A_7 A_8$
	$A_1 A_2$		$A_5 A_6$
	$A_1 A_3$		$A_5 A_7$
Possible gametes	$A_1 A_4$		$A_5 A_8$
	$A_2 A_3$		$A_6 A_7$
	$A_2 A_4$		$A_6 A_8$
	$A_3 A_4$		$A_7 A_8$

There are 36 possibile genotypes that can be obtained in the F_1 generation. However, all possible genotypes are tetrallelic. Therefore, if the breeder is searching for just a monoallelic combination (i.e., an individual with only one type of allele), such genotype will not be found after only one generation of random mating F_1 plants. This is another distinctive autotetraploid characteristic: all possible genotypes from a cross (monoallelic, diallelic, triallelic and tetrallelic) are produced only after two generations of random mating (Table 6).

As presented in Table 6, the frequency of the monoallelic class is very low, which constitutes another autotetraploid characteristic. In addition, it can be noticed that equilibrium among these different classes is only obtained after four generations of random mating.

Table 6. Genetic frequency and genotypic structure of an autotetraploid population along different generations.

Generation	Structure and frequencies			
	Monoallelic	Diallelic	Triallelic	Tetrallelic
F_1	0.000	0.000	0.000	1.000
F_2	0.000	0.037	0.426	0.537
F_3	0.001	0.074	0.474	0.450
F_4	0.002	0.106	0.492	0.410

Source: Adapted from Busbice et al. (1972).

Gains from selection

To develop superior genotypes, it is necessary to combine a number of favorable traits that allow not only higher yields *per se* but also other characteristics related to the satisfaction of market quality requirements. Thereby, selection based on only one or just a few traits seems inadequate, since it is going to lead to a final product that will be superior only on the selected traits (Cruz; Regazzi, 1997). This is very important in alfalfa improvement as a feed, because the final goal is not only to increase forage yield but mainly to improve forage quality and animal intake.

Response to selection

The need to perform selection for several traits in order to simultaneously improve all of them requires the use of a selection criteria based not purely on one trait or on indirect selection alone. Hill (1971) compared response to selection between diploid and autotetraploid populations, concluding that response was faster in the former that in the latter.

To illustrate and to compare the gain from selection between diploid and and in autotetraploid populations, an example in which the allele to be selected is dominant (A > a) and the gene under consideration is in equilibrium in both populations is analyzed.

If $f(A) = p$ and $f(a) = q$, then $P(A) = p$ and $P(a) = q$, in which $p + q = 1$.

The predicted genotypic ratio is given by the equation $(p + q)^4$ and it will be:

$(p + q)^4 = p^4$ AAAA: $4p^3q$ AAAa: $6p^2q^2$ AAaa: $4pq^3$ Aaaa: q^4 aaaa

Since selection will eliminate the aaaa genotype, then the frequency of p becomes p', and the effect from selection (Δp) will be:

$\Delta p = p' - p,$

in which

$$p' = \frac{p^4 + 4p^3q + 6p^2q^2 + 4pq^3}{4(p^4 + 4p^3q + 6p^2q^2 + 4pq^3)} = \frac{p}{1 - q^4}$$

Thus, selection gain will be:

$$\Delta p = p' - p = \frac{p}{1 - q^4} - p = \frac{pq^4}{1 - q^4}$$

According to the previous equation, Δp, which expresses the frequency variation for the selected allele, is a function of its initial frequency.

In the case of a diploid population, Δp is estimated by the following equation:

$$\Delta p = p' - p = \frac{p}{1 - q^2} - p = \frac{pq^2}{1 - q^2}$$

Figure 1 shows the frequency variation for allele A, as a response to selection, relative to its initial frequency (p) in both diploid and autotetraploid populations. It can be noticed that the rate of the variation in the tetraploid population is much slower than in the diploid population, which explains the longer time usually required to promote genetic changes in tetraploid species.
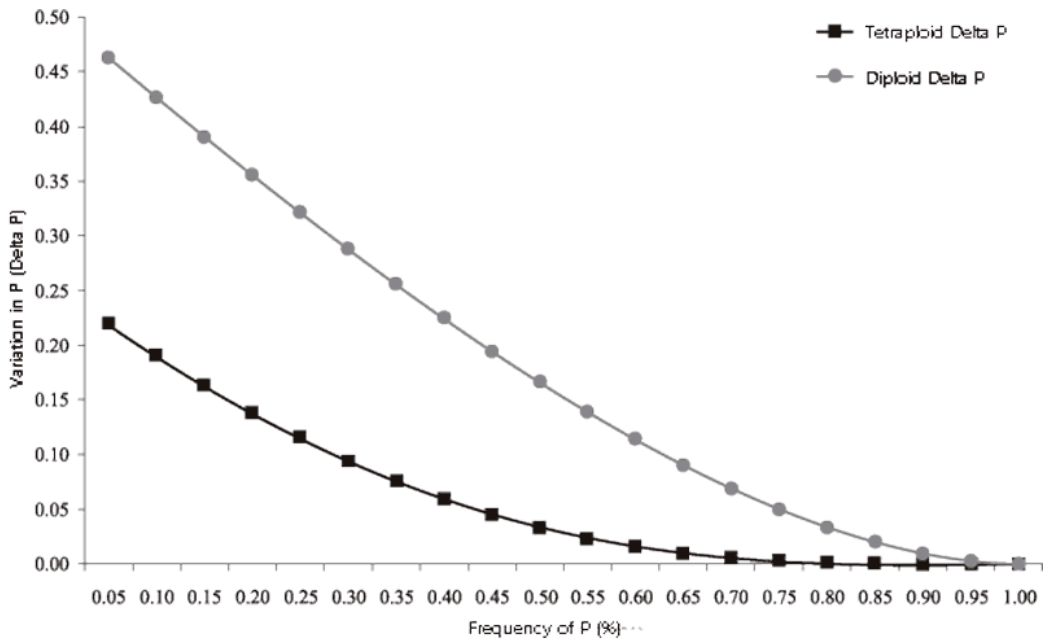
Figure 1. Response to selection as variation of allelic frequency relative to the initial frequency of the allele (*p*) in both diploid and tetraploid populations.
Source: Hill (1971).

Components of genetic variance and heritability

Success in improving any trait necessarily requires that the trait is inheritable and that there is sufficient genetic variation in the population that makes selection possible. In this section, to study the inheritance and the variation of quantitative traits it will be considered the basic model $P = G + E$, in which the phenotypic value of an individual (P), results from the action of the genotype (G) and the influence of the environment (E). Similarly, the phenotypic variance (σ_P^2) is the result of the genotypic variance (σ_g^2) and the environmental variance (σ_E^2).

Kempthorne (1955) demonstrated that in autotetraploid populations, as in the case of alfalfa, genotypic variance (σ_g^2) can be decomposed in:

σ_G^2: genotypic variance of the population
σ_A^2: additive variance
σ_D^2: digenic variance
σ_T^2: trigenic variance
σ_Q^2: quadrigenic variance

These variances are obtained from the genotypic value $V(G)$ which, for a given individual, is given by the equation:

$$V(G) = A_i A_j A_k A_l = \mu + \alpha_i + \alpha_j + \alpha_k + \alpha_l + \beta_{ij} + \beta_{ik} + \beta_{il} + \beta_{jk} + \beta_{jl} + \beta_{kl} + \gamma_{ijk} + \gamma_{ijl} + \gamma_{jkl} + \delta_{ijkl}$$

While A_i, A_j, A_k and A_l are the alleles from a particular locus; μ is the mean of the population at genetic equilibrium.

The other terms in the equation express the effects that cause deviations of each individual from the population mean (μ). In the equation, the variance components σ_A^2, σ_D^2, σ_T^2 and σ_Q^2 are represented by α, β, γ and δ, respectively.

According to Rumbaugh et al. (1988), additive individual (α_{i-l}) effects in tetraploids are the same as additive effects in diploid models. Likewise, digenic effects (β_{ij-kl}) are analogous to the heterotic effects in diploid organisms. However, trigenic ($\gamma_{ijk-jkl}$) and quadrigenic (δ_{ijkl}) effects have no analogy in diploid models.

In alfalfa, the estimation of the genetic variance based on covariance between relatives has been proposed, as well as a procedure for estimating genetic variance components (Levings; Dudley, 1963). For the latter, it was suggested the use of a partial diallel cross design together with parent-offspring regression and the estimation of genotypic variance among clones. This scheme was used by Dudley et al. (1969) to calculate the variance components related to dry matter and plant size in alfalfa. It was concluded that while trigenic and quadrigenic effects had a relative importance, additive and digenic effects were the most and the least important, respectively.

The establishment of genetic models is very important for estimating genetic variance and covariance components, as well as interactions with the environment. These parameters, in turn, have great implications on the estimation of heritability, both in broad and narrow sense.

Only the phenotypic value of an individual can be directly measured; however, it is just the genetic value will influence over the next generation. Therefore, it is necessary to estimate the proportion of the total variability existing in the population which is genetic in nature.

Heritability expresses the proportion of genetic variation relative to phenotypic variation, i.e., the relationship between genetic variance and phenotypic variance in the selection units (Cruz, 2005). The previous concept implies that heritability is a function of the type of selection that is being performed, whether it is selection among families, selection among individuals, stratified mass selection, or any other type of selection. Heritability also depends on the experimental design and the estimation method that are used, the trait under study, and the genetic diversity of the population, among other factors.

Heritability can be calculated in a broad or in a narrow sense, taking into account genetic variance or just additive variance, respectively. Heritability in broad sense can be estimated by the following equation:

$$h_b^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

where:

h_b^2 = heritability in broad sense

σ_g^2 = genetic variance of the selection unit

σ_p^2 = phenotypic variance selection unit

Kehr and Gardner (1960), using progenies from a polycross and clones of the parental genotypes, estimated heritability through the following equation:

$$h^2 = \frac{4\sigma_{px}^2 + 2COV_{op}}{2\sigma_c^2}$$

in which:

h^2 = heritability

σ_{px}^2 = variance among progenies from polycross

COV_{op} = covariance between parental clones and progenies from polycross

σ_c^2 = phenotypic variance among clones

Once the heritability value was calculated, the gain from selection can be estimated. The possibility to predict the gain from a given selection strategy constitutes one of the main contributions of quantitative genetics to breeding. The use of this information allows not only to more effectively conduct the improvement program, but also to predict the progress (gain) from selection.

Gain from selection (GS) can be estimated by the equation:

$$GS = SD \times h^2$$

where h^2 is the heritability values and SD is the selection differential, i.e. the difference between the mean of the selected population and the mean of the original population. For instance, if the mean yield of the original is 15 ton ha^{-1} year^{-1} and the mean yield of the selected population is 18 ton ha^{-1} year^{-1}, the SD would be 3 ton ha^{-1} year^{-1}; and if h^2 is 0.50, then the GS will be:

$$GS = 3 \times 0.5 = 1.5 \text{ t ha}^{-1} \text{ year}^{-1}$$

Inbreeding

Inbreeding is the phenomenon which happens as a result of mating related individuals – in other words, sharing common ancestors. The inbreeding

coefficient, represented by *F*, refers to the probability that the alleles of a gene in an individual are identical by descent – that is, the alleles could have been derived by replication of an allele found in a common ancestor.

The main effect of inbreeding is to increase the frequency of homozygotes at all loci in the population. However, in the absence of selection, inbreeding alone does not change allele frequencies; it only alters the arrangement of alleles in the genotypes of the population. Thus, under no selection, this allele reorganization is just a short-lived change: homozygote frequency will decrease as soon as the mating system changes.

According to Wrigth (1922), inbreeding results from the union of identical gametes and it is expressed by the correlation between the values of the gametes that form the progeny from a population.

Considering a particular locus of an individual X which produces a gamete ab, the value of the inbreeding coefficient (*Fx*) for that individual will be given by $Fx = P(a \equiv b)$, where ≡ means being identical by descent. Thus, the inbreeding coefficient for a particular individual is equivalent to the probability to which this individual will produce gametes that are identical by descent.

If individual X is crossed to an individual Y, which produces gametes "ef", their offspring (F_1) will have the following inbreeding coefficient:

$$F_{1xy} = 1/6 \, [P(a \equiv b) + P(a \equiv e) + P(a \equiv f) + P(b \equiv e) + P(b \equiv f) + P(e \equiv f)]$$

If r_{xy} is defined as the probability for a random allele from X to be identical by descent to a random allele from Y, then:

$$P(a \equiv e) = P(a \equiv f) = P(b \equiv e) = P(b \equiv f) = r_{xy}$$

Thus:

$$F_{1xy} = 1/6 \, (4r_{xy} + F_x + F_y) = 2/3 \, r_{xy} + 1/6 \, (F_x + F_y)$$

Therefore, in an autotetraploid the progeny can be inbred either when the parents are related or when they are inbred. When parents are not related, the offspring always inherits 1/3 of the parental mean of inbreeding. This is a consequence of the diploid gametes produced by autotetraploids. In this context, if it is assumed that X is an inbred individual with its four alleles identical by descent ($a \equiv b \equiv c \equiv d$), Y is not inbred ($e \neq f \neq g \neq h$), and X and Y are not related, the progeny (Z), from the union of gametes "ab" and "ef" will have the following genotypic constitution: $a \equiv b \neq e \neq f$. If *F* is the probability of alleles being identical by descent, then:

$$F_z = 1/6 \, [P(a \equiv b) + P(a \equiv e) + P(a \equiv f) + P(b \equiv e) + P(b \equiv f) + P(e \equiv f)] =$$

$$= 1/6 \, (1 + 0 + 0 + 0 + 0 + 0) = 1/6$$

Since the mean inbreeding of the parents is (1 + 0) / 2 = ½, and one third of this mean is equal to 1/6, which is another way to estimate the inbreeding value of the offspring from crossing ab x ef parents.

The above has practical consequences for alfalfa breeding and the development of hybrids and synthetic varieties, because the use of parents which are not hybrid nor inbred produce non-hybrid progenies.

The inbreeding coefficient can be used to compare different breeding methods for producing alfalfa varieties. To illustrate this comparison, let us consider three hypothetical breeding schemes involving four parental individuals (A, B, C and D):
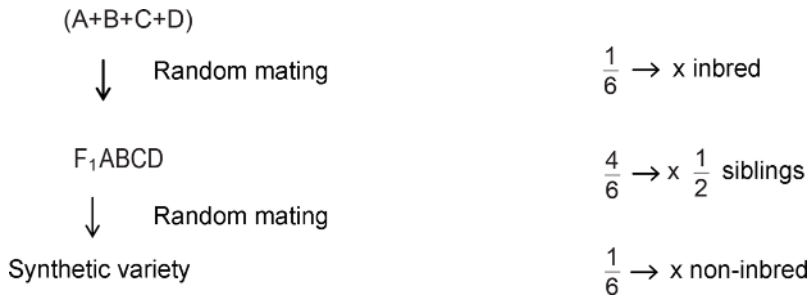
1) Production of hybrid through double crossing in two generations:

A x B   C x D   generation 1

↓   ↓

F_1AB F_1CD generation 2

X

Hybrid
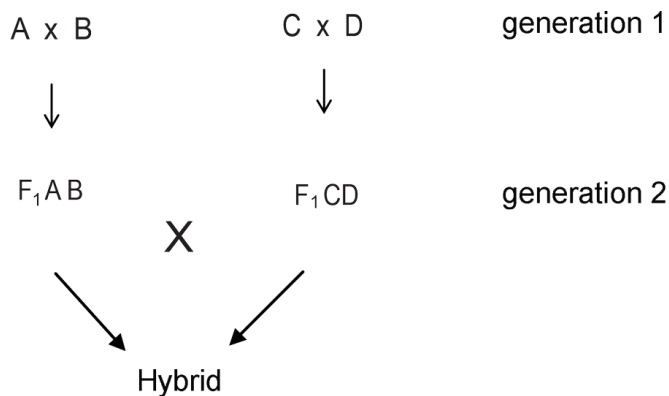
2) Production of a synthetic variety through random mating of two F_1 individuals originated from different parents:
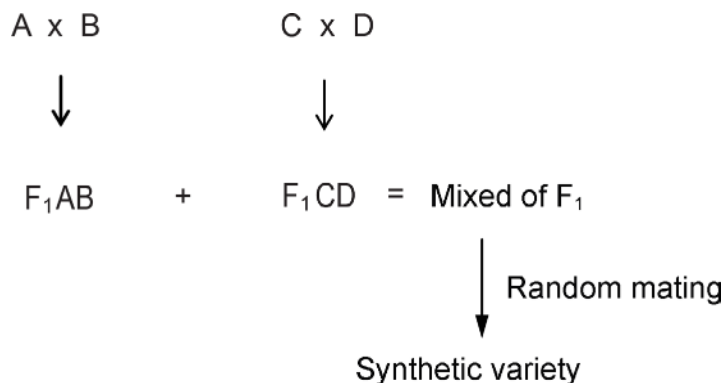
A x B   C x D

↓   ↓

F_1AB + F_1CD =   Mixed of F_1

Random mating

Synthetic variety

3) Production of a synthetic variety through random mating of the four parents:

(A+B+C+D)

↓ Random mating

$\frac{1}{6}$ → x inbred

F$_1$ABCD

↓ Random mating

$\frac{4}{6}$ → x $\frac{1}{2}$ siblings

Synthetic variety

$\frac{1}{6}$ → x non-inbred

Assuming that A, B, C and D are not related and that they have a similar degree of inbreeding, identified as F_0, probabilities for gamete formation would be as follows:

- For case 1 (hybrids), tha gametes from parent A (a_1 a_2 a_3 a_4) and B (b_1 b_2 b_3 b_4), having four alleles each, will have the structure and frequencies shown in Table 7.

In the A X B cross, 36 different possible genotypes will be generated. The same will occur for the C X D cross. If the hybrid were produced by crossing parents with two alleles each, as for example a_1a_2 b_1b_2 x c_1c_2 d_1d_2, then the probability for the different gametes would be as shown in Table 8.

Table 7. Probability of occurrence of the different gametes produced by the unrelated parents A and B.

Gametes from A	Frequency	Gametes from B	Frequency
a_1a_2	1/6	b_1b_2	1/6
a_1a_3	1/6	b_1b_3	1/6
a_1a_4	1/6	b_1b_4	1/6
a_2a_3	1/6	b_2b_3	1/6
a_2a_4	1/6	b_2b_4	1/6
a_3a_4	1/6	b_3b_4	1/6

Table 8. Probability of occurrence of the different gametes produced by four unrelated parents (A, B, C and D) each one having two alleles (1 and 2).

Gametes from A and B	Frequency	Gametes from C and D	Frequency
$a_1 a_1$	1/6	$c_1 c_1$	1/6
$a_1 a_2$	1/6	$c_1 c_2$	1/6
$a_2 a_2$	1/6	$c_2 c_2$	1/6
$b_1 b_1$	1/6	$d_1 d_1$	1/6
$b_1 b_2$	1/6	$d_1 d_2$	1/6
$b_2 b_2$	1/6	$d_2 d_2$	1/6

Since

$P(a_i \equiv a_{j\prime}) = F_A = F_0$
$P(b_i \equiv b_{j\prime}) = F_B = F_0$
$P(c_i \equiv c_j) = F_c = F_0$
$P(d_i \equiv d_j) = F_d = F_0$
$P(a_i \equiv b_j) = P(a_i \equiv c_j) = P(a_i \equiv d_j) = P(b_i \equiv c_j) = P(b_i \equiv d_j) = P(c_i \equiv d_j) = 0.$

and considering the four possible genotypes that may receive two alleles from the same parent (i.e., $a_1\ a_2\ c_1\ c_2$; $a_1\ a_2\ d_1\ d_2$; $b_1\ b_2\ c_1\ c_2$ e $b_1\ b_2\ d_1\ d_2$), then the value of F in each case can be calculated. For example, for the case of $a_1\ a_2\ c_1\ c_2$, it would be:

$F = 1/6\ [P(a_1 \equiv a_2) + P(a_1 \equiv c_1) + P(a_1 \equiv c_2) + P(a_2 \equiv c_1) + P(a_2 \equiv c_2) + P(c_1 \equiv c_2)]$
$F = 1/6\ (F_A + F_c) = 1/6\ (2F_0) = 1/3\ F_0.$

Likewise, the coefficient of inbreeding for the 16 genotypes which received two alleles from the same parent ($a_1\ a_2\ c_1\ d_1$, $a_1\ a_2\ c_1\ d_2$, $a_1\ a_2\ c_2\ d_1$, $a_1\ a_2\ c_2\ d_2$, ... , $a_2\ b_2\ d_1\ d_2$) is obtained the same way. For example, for $a_1\ a_2\ c_1\ d_1$ it will be:

$F = 1/6\ [P(a_1 \equiv a_2) + P(a_1 \equiv c_1) + P(a_1 \equiv d_1) + P(a_2 \equiv c_1) + P(a_2 \equiv d_1) + P(c_1 \equiv d_1)]$
$F = 1/6\ (F_A) = 1/6\ F_0.$

For the remaining genotypes, the value of F will be zero.

Therefore, the mean of the inbreeding coefficient for the hybrid produced by this cross will be given by:

$$\frac{4}{36} \times \frac{1}{3} F_0 + \frac{16}{36} \times \frac{1}{6} F_0 + \frac{16}{36} \times 0\ F_0 = \frac{1}{9} F_0$$

Since it is expected that the other possible crosses be originated by individuals bearing the same characteristics as the ones presented before, it can be inferred that the mean of inbreeding must be repeated in all these crosses; thus, the inbreeding coefficient in the resulting hybrid will be given by $1/9 \, F_0$.

- For cases 2 (synthetic variety from random mating on two F_1 individuals originated from different parents) and 3 (synthetic variety from random mating of the four parents), the value of F can be estimated using the same logic as in the previous case, being as a consequence:

$$F = \frac{1}{24} + \frac{17}{72} F + s \left[\frac{13}{17} + \frac{5}{24} F_0 + s \left(\frac{1}{12} + \frac{1}{4} F_0 \right) \right]$$

where s represents the frequency of self-fertilization (selfing) within the crosses.

Based on the results from all previous equations, it can be concluded that the coefficient of inbreeding is lower in hybrids than in synthetic varieties. Of course, this is an expected outcome since crosses between related parents were not allowed during the analyzed examples. In the same way, it can be observed that in synthetic varieties developed either by one or two random mating generations the coefficients of inbreeding will have similar values.

Heterosis and inbreeding depression

Alfalfa is very susceptible to inbreeding depression. Tysdal et al. (1942) estimated a 78% reduction in forage yield and 92% in seed production after eight generations of self-fertilization. On the other hand, heterosis also occurs in alfalfa. Demarly (1963) stated that simple, triple (3-way cross) and double hybrids produced 38%, 39% and 45% more forage than the original populations.

Rotili (1970) reported that after three generations of self-fertilization together with selection for vigor, the inbreeding depression in the progenies was significantly reduced. This is attributed to the assumptions that selection makes possible to either maintain heterozygosity or increase the frequency of favorable genes and unknown gene combinations.

Determining the effective degree of inbreeding within the breeding program, in order to favor genetic gains from the parental population, is one of the important objectives for alfalfa breeders. This can be possible when the greatest expression of heterosis in hybrids and synthetic varieties is obtained by the combination of inbreeding and selection.

Demarly (1963) stated that the genome of a tetraploid individual can be characterized by the relative proportion of tetragenic, trigenic, digenic,

simplex and nulliplex genetic constitutions. In this context, the study proposed to estimate the relative proportion of each structure in every generation within a controlled mating system. Complementarily, it was concluded that the initial genetic constitution is very important in explaining heterosis and inbreeding depression.

Dudley (1964) reported that from all possible simple and double mating combinations between quadruplex, triplex, duplex, simplex or nulliplex individuals, in addition to their S_1, S_2, S_3 and their homozygote progenies, maximum heterosis from both dominance and overdominance will be found not only in simple parental crosses, but also in progeny crosses; on the other hand, the effect of selection among crosses will be not very important.

Several mathematical models have been proposed for explaining inbreeding depression and heterosis in autotetraploids. One of these models, suggested by Busbice and Wilsie (1966), the genotypic structures at one locus are given by the proportion of T_0 (quadruplex), T_1 (triplex), T_2 (duplex), T_3 (simplex) and T_4 (nulliplex) constitutions, with frequencies P_0, P_1, P_2, P_3, and P_4, respectively. Thus, the genotypic value for one single locus or for a specific chromossome segment will be given by the individual value of the alleles, as well as by the value of two, three and four alleles, as follows:

$GV_{T0} = GV_{iiii} = i + i + i + i + 6(ii) + 4(iii) + 1(iiii) = 4(i) + 6(ii) + 4(iii) + 1(iiii)$.

Thus:

$GV_{T1} = GV_{iiij} = [3i + 1j] + [3(ii) + 3(ij)] + [1(iii) + 3(iij)] + (iiij)$,

$GV_{T2} = GV_{iijj} = [2i + 2j] + [1(ii) + 4(ij) + 1(jj)] + [2 (iij) + (2(ijj)] + 1(iijj)$,

$GV_{T3} = GV_{iijk} = [2i + j + k] + [1(ii) + 2(ij) + 2(ik) + (jk)] + [1(iij) + 1(iik) + 2(ijk)] + 1(iijk)$

and

$GV_{T4} = GV_{ijkl} = [i + j + k + l] + [1(ij) + 1(ik) + 1(il) + 1(jk) + 1(jl) + 1(kl)] + [1(ijk) +$
$+ 1(ijl) + 1(ikl) + 1(jkl)] + 1(ijkl)$,

where

GV = genotypic value of the structure
i, j, k, l = additive values of each allele, separately
ii, ij, ik, il, jk, jl and kl = values of the first-order interactions
iii, ijl, il and jkl = values of the second-order interactions
$iiii$, $iiij$, $iijj$ and $ijkl$ = values of the third-order interactions

According to Busbice and Wilsie (1966), the mean of the population is given by the total of additive and interaction values of genes over all loci and all individuals in a given population. Then, they proposed that all the additive

values of the genes are equal to the population mean (assuming inbreeding to homozygosity and no selection), which is represented by the term A. Since heterosis results from heterogenic interaction between non-identical alleles, they suggested the estimation of the average genotypic values as follows:

GV_{iiii} = A

GV_{iiij} = A + ij

GV_{iijj} = A + ij

GV_{iijk} = A + ij + ik + jk + ijk

GV_{ijkl} = A + ij + ik + il + jk + jl + kl + ijk + ijl + ikl + jkl + ijkl.

The genotypic value of the population (GV_{pop}) will be given by:

$$GV_{pop} = A + (P_1 + P_2 + 3P_3 + 6P_4) (ij) + (P_3 + P_4) (ijk) + P_4 (ijkl),$$

where:

ij, *ijk* and *ijkl* = nonallelic interactions of first, second and third order, respectively.

Based on the genotypic values, it can be verified that genotypes with tetragenic and trigenic structures are more important in the expression of heterosis in alfalfa. Busbice and Wilsie (1966) stated that the proportion of the different structures is affected by the generation of inbreeding, and that these changes could be calculated as the sum of all structures in the theoretical genotypic formations of the inbred progenies. By considering each of the genotypic structures separately, they were able to associate the loss of interactions between non-identical alleles to yield, as well as to the inbreeding coefficient. These authors also observed that inbreeding depression in alfalfa is related to the rate by which first-order interactions are lost from tetragenic and trigenic loci. The effect of losing interactions from digenic loci is not sufficiently fast to explain inbreeding depression.

The previous genetic model and the one presented by Gallais (1967) have provided certain insight on inbreeding, selection and hybridization in alfalfa, even though only for carefully planned experiments. Alfalfa is very sensitive to inbreeding, so that any process which increases inbreeding in the population will consequently lead to a reduction of heterosis together with the emergence of inbreeding depression.

Using a double-cross scheme, Bingham (1979) proposed to cross at least four selected and unrelated cultivars in order to reach maximum heterosis. At the third generation of random mating, 50% of the individuals in the resulting population should be in theory the product of double crossing, thus representing maximum heterosis.

Autotetraploid model: implications on breeding

The topics on quantitative genetics previously presented have implications on alfalfa genetic improvement. In alfalfa, the production of all possible genotypes from a given cross makes necessary to allow at least two generations of random mating, and not only one generation as is the case for diploids. The frequency of extreme genotypes (like nulliplex or quadruplex) in the population is low; therefore, if the breeder is looking for such genotypes, it will be necessary to assess a large number of individuals in order to increase the probability for detecting them.

Another important characteristic of autotetraploids is that they reach gametic equilibrium in an asymptotic way, because the diploid nature of their gametes does not allow the production of all possible genotypes in just one generation of random mating, as is the case for diploids. Generally, equilibrium is reached after four or five generations of random mating (Busbice et al., 1972).

The sensibility of alfalfa to inbreeding has an impact on predicting the yield of synthetic varieties in advanced generations (Busbice; Gurgis, 1976). Thus, breeders should always consider that: 1) self-fertilization followed by selection as a breeding method can be a problem, making the production of pure lines and the development of inbred lines for obtaining hybrids non-practical; and 2) the use of non-related and non-inbred parents should be always taken into account for producing non-inbred progenies with lower vigor and yield reduction.

In case of selection for increasing resistance to pests and diseases, which is usually conditioned by either one or a few genes, response to selection is fast until the frequency of such gene reaches 0.5; after that, response to selection becomes slow and difficult to verify. This is due to the fact that if the frequency of a dominant allele is 0.5, then nearly 93% of the individuals in the population will express this phenotype (Rodriguez, 1986).

Final considerations

The existence of self-incompatibility and self-sterility mechanisms in alfalfa favors cross-pollination. However, the autotetraploid nature of alfalfa has deep implications on the genetic behavior and the genotypic structure of the populations. Effects on segregation and gamete formation, estimation of variance components, gain from selection and production of at least two generations of random mating to obtain all possible genotypes from a cross, are

particularly important. Additionally, alfalfa manifests a pronounced inbreeding depression, which conditions the breeding methods to be used and highlights the importance of using unrelated parents.

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