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Mating Systems and Inbreeding Pressure in Populations of Wild Lentil Tare, *Vicia tetrasperm* (Leguminosae)

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The mating systems of natural populations of *Vicia tetrasperm* in Korea were determined using allozyme analysis. The result suggests that *V. tetrasperm* is low rates of outcrossing or mix-mating outcrossing (self-fertilization, $s < 0.5$). At the population levels, the values of inbreeding coefficient of ten populations in Korea varied from 0.131 to 0.176, giving an average 0.154. For ten natural populations, multi-locus estimates of outcrossing (tm) was 0.333 across fifteen polymorphic loci, with individual population values ranging from 0.269 to 0.423. The differences between the tm and ts values were not close to zero ($tm - ts > 0.154$), indicating that biparental inbreeding was significant in the loci. The reason for relatively low outcrossing rates of some populations could be attributed to extensive consanguineous mating and isolation of flowering mature plants. Although heterozygote excess was observed in one natural population, most populations exhibited varying degrees of inbreeding and heterozygotes deficit. Thus, selection against homozygotes operated in the progeny populations throughout the life cycle.

Key words : Allozyme analysis, mating systems, *Vicia tetrasperm*

Introduction

The genus *Vicia* comprises ca. 140 species, widely distributed most commonly in temperate regions of Europe, Asia and the America [24]. *Vicia tetrasperm* Schreb. (Leguminosae) is mostly distributed in the temperate regions of both hemispheres. It is typically found in low mountain regions in Korea and Japan, where grows at elevations as low as 300 m above sea level. In addition, the wild lentil tare plants grow in fallow fields, under hedgerow, and along roadsides and riverbanks, and often colonizing nearly available sites. Populations of *V. tetrasperm* are typically small and distributed in patches. Many studies have been conducted for the cultivated faba bean *V. faba* [1,12,16,21,22], but there are only a few isozyme studies in other lentil tare species [10,27,28]. Most of the isozyme analyses of *Vicia* have been used in evolutionary and taxonomic studies [27,28], inbred-line recognition [2,7], and outcrossing rate estimation [21]. Although molecular and biochemical approaches are now increasingly being applied to address the taxonomic and phylogenetic relationships within the subgenus *Vicia* [1,6,15,27], no population genetics studies have been conducted, especially on the mating system of lentil tare species.

V. tetrasperm is a profusely flowering annual, with autogamous magenta flowers that are occasionally visited by some insect species [8]. The objectives of this study were to estimate the level of genetic diversity in the species, to describe how its genetic variation is distributed within and among its populations.

Allozyme variation within plant populations often shows structured pattern in space, presumably reflecting kinship structure that has arisen through by distance due to restricted dispersals [4]. Once kinship structure is established, restricted seed and pollen dispersals lead to outcrossing between genetic relative, a phenomenon known as biparental inbreeding [23]. Thus, even populations of obligate outcrossers may routinely experience some level of inbreeding [4]. In addition, selfing rate, inbreeding depression, and relative fecundity have been estimated at different life history stages [11].

The mating system is dynamic and can vary in space and time [10]. For instance, Murawski et al. [13] reported that population and individual outcrossing estimates were associated with tree density or degree of spatial isolation. Maki [11] reported the selfing rate or inbreeding in gynodioecious population of *Chionographis japonica* var. *kurohimensis*. In this study I investigated the mating system of ten natural populations of *V. tetrasperm* in Korea using allozyme markers.

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Materials and Methods

Sampling Procedure

V. tetrasperm (Leguminosae) was collected from ten populations in Korea and Japan (Fig. 1). Forty-five to 60 plants were sampled from each population. Plants were determined using a tape measure and compass in relation to a lattice of reference points that were established throughout the study area. One leaf per plant was sampled during the period from 2006 to 2007. The distance between selected individuals was about 5 m in order to avoid including individuals with common lineage. Leaves gathered from natural populations were stored in plastic bags for several days in a refrigerator until electrophoresis was carried out.

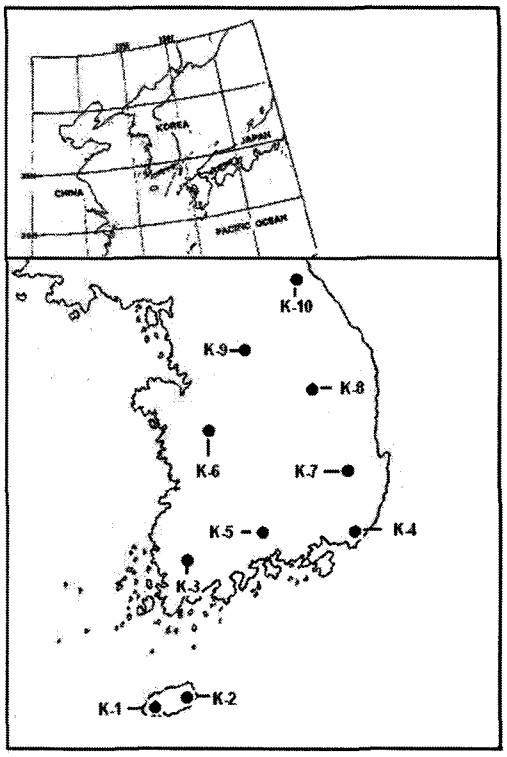

Fig. 1. Collection localities for populations of *Vicia tetrasperm* as sources for mating systems by allozyme analysis. K-1: Donggwang-ri, Namjeju-gun, Jeju-do; K-2: Seongsan-eup, Namjeju-gun, Jeju-do; K-3: Hwasun-eup, Hwasun-gun, Jeollanam-do; K-4: Hoedong-dong, Geumjeong-gu, Busan Metropolitan City; K-5: Yangbo-myeon, Hadong-gun, Gyeongsangnam-do; K-6: Osan-ri, Yangcheon-myeon, Chungcheongnam-do; K-7: Bugan-myeon, Yeongcheon-si, Gyeongsangbuk-do; K-8: Gamcheon-myeon, Yecheon-gun, Gyeongsangbuk-do; K-9: Geumsa-myeon, Yeoju-gun, Gyeonggi-do; K-10: Seo-myeon, Yangyang-gun, Gangwon-do.

Enzyme electrophoresis

Approximately 300-500 mg leaf tissues were ground with a cold mortar and pestle in 300-500 μl of extraction buffer (0.1% 0.05 ml β-mercaptenthanol, 0.001 M EDTA, 0.01 M potassium chloride, 0.01 M magnesium chloride hexahydrate, 45 w/v 1g PVP, 0.10 M Tris-HCl buffer, pH 8.0).

Electrophoresis was performed using 12.0% starch (Sigma Co.) gels according to the methods by Soltis et al. [20]. A total of twelve enzyme systems were assayed for this study: acid phosphatase (ACP), esterase (EST), glutamate oxaloacetate transaminase (GOT), phosphoglucoisomerase (PGI), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), peroxidase (PER), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), and superoxide dismutase (SOD).

Isozyme bands were assigned to putative loci based on analysis of observed variation patterns. For example, the most anodal isozyme locus was arbitrarily designated '1' and subsequent isozymes were assigned sequentially higher numbers. Likewise, alleles at each locus were designated sequentially with the most anodally migrating allozyme designated 'a' and progressively slower forms 'b', 'c', and so on. All *V. tetrasperm* allozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most other allozyme studies of plants, as documented by Weeden and Wendel [25].

Data analysis

A locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. The multilocus outcrossing rates (*t*m) and mean single locus outcrossing rates (*t*s) were calculated by multilocus mixed mating program (MLT) [17]. MLT is based on the multilocus outcrossing estimation procedure of Ritland and Jain [18], which assumes that progeny are derived from either random mating (outcross) or self-fertilization. The differences between the two values (*t*m - *t*s) indicates the magnitude of the biparental inbreeding (*F*bi).

The observed inbreeding coefficient (*F*in) of Wright [26] was calculated for natural populations as *F*in = 1 - (*Ho*/*He*), where *Ho* is the observed heterozygosity, *He* = 1 - pi^2 is the expected heterozygosity under random mating, and *pi* is the frequency of the *i*th allele [10]. The observed heterozygosity was compared with Hardy-Weinberg expected values using Wright's fixation index [26]. These in-

dices were tested for deviation from zero by χ^2-statistics fellowing Li and Horvitz [9].

Results

At the species level, sixteen of the 32 loci (50.0%) showed detectable polymorphism in at least one population. The remaining sixteen loci (*Acp-4, Got-1, Idh-1, Lap-1, Lap-2, Mdh-2, Mdh-4, Mdh-5, Per-3, Per-4, Pgd-1, Pgd-2, Pgi-1, Pgm-1, Sod-2* and *Skd-1*) were monomorphic in all populations. They omitted in this study because they are not contributed the estimation of mating system analyses.

Multilocus outcrossing rates (*t*m) within loci was 0.333 with individual population values ranging from 0.269 for population K-5 to 0.423 for population K-10 (Table 1). There are not significant differences among populations ($p > 0.05$). The mean single locus outcrossing rates (*t*s) varied between 0.079 (K-10) and 0.110 (K-4), giving an average of 0.096 over all polymorphic loci. The mean biparental inbreeding coefficient (*F*bi) was 0.237. The differences between the *t*m and *t*s values were not close to zero (*t*m - *t*s > 0.154), indicating that biparental inbreeding was significant in the loci.

Table 1. Multilocus (*t*m) and mean single locus (*t*s) outcrossing rates, and the biparental inbreeding coefficients (*F*bi) estimated from outcrossing rates in ten populations of *V. tetrasperm*. The minimum and maximum values appear in parentheses

Pop.	*t*m	*t*s	*F*bi (*t*m - *t*s)
K-1	0.351 (0.127-0.576)	0.089 (0.001-0.243)	0.262
K-2	0.279 (0.084-0.684)	0.104 (0.001-0.292)	0.175
K-3	0.305 (0.119-0.538)	0.105 (0.015-0.284)	0.200
K-4	0.337 (0.085-0.693)	0.110 (0.014-0.251)	0.227
K-5	0.269 (0.000-0.608)	0.100 (0.035-0.191)	0.169
K-6	0.330 (0.000-0.739)	0.102 (0.005-0.207)	0.228
K-7	0.349 (0.103-0.709)	0.087 (0.027-0.222)	0.262
K-8	0.355 (0.155-0.595)	0.124 (0.000-0.314)	0.231
K-9	0.327 (0.121-0.567)	0.086 (0.000-0.225)	0.241
K-10	0.423 (0.096-1.137)	0.079 (0.003-0.186)	0.244
Mean	0.333 (0.208-0.496)	0.096 (0.035-0.178)	0.237

For ten natural populations, the inbreeding coefficient (*F*in) was 0.154 across 15 polymorphic loci, with individual population values ranging from 0.131 for population K-9 to 0.176 for population K-4 (Table 2). The *F*in values were significantly heterogeneous on many locus including *Acp-3, Est-1, Idh-2, Mdh-3, Per-2,* and *Sod-3.*

Tests for deviations from mating system equilibrium revealed somewhat different results in all natural populations (Table 3). Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations. Wright's fixation indices for polymorphic loci were positive in most cases for ten natural populations (228/229), and 93.0% of those (212/228) departed significant from zero. Only one of indices were negative, indicating an excess of heterozygosity on *Skd-2* populations K-10, however, none was departed significant from zero ($P < 0.05$).

Discussion

Presence or absence of self-incompatibility mechanisms, availability of pollinators and their foraging behavior, and flower density and phonological variation are among the factors that affect the mating system [3,26,27]. The mean value of *t*m of ten populations in Korea was 0.333. Some species have a higher *t*m than that observed in Korean *V. tetrasperm* populations, e.g. *Phaseolus coccineus* (*t*m = 0.592-0.698) [3], *Senna multijuga* (*t*m = 0.540-0.838) [19].

Table 2. Inbreeding coefficient (*F*in) for ten populations of *V. tetrasperm*

Pop.	*Acp-1*	*Acp-2*	*Acp-3*	*Est-1*	*Est-2*	*Got-2*	*Idh-2*	*Mdh-1*	*Mdh-3*	*Per-1*	*Per-2*	*Pgi-2*	*Skd-2*	*Sod-1*	*Sod-3*	Mean
K-1	0.077	0.429	0.237	0.121	0.146	0.186	0.071	0.319	0.206	0.063	0.049	0.239	0.129	0.152	0.063	0.166
K-2	0.095	0.289	0.303	0.065	0.136	0.156	-	0.380	0.156	0.031	0.026	0.292	0.086	0.059	0.056	0.142
K-3	0.121	0.048	0.162	0.188	0.149	0.162	0.333	0.275	0.188	0.056	0.057	0.304	0.138	0.178	0.027	0.159
K-4	0.167	0.122	0.108	0.161	0.150	0.200	0.361	0.293	0.125	0.143	0.100	0.237	0.188	0.250	0.030	0.176
K-5	0.103	0.139	0.154	0.219	0.122	0.163	0.091	0.310	-	0.080	0.229	0.293	0.148	0.157	0.130	0.156
K-6	0.333	0.139	-	0.303	0.119	0.133	0.180	0.278	-	0.051	0.159	0.244	0.222	0.128	0.111	0.160
K-7	0.051	0.219	-	0.138	0.150	0.119	0.348	0.158	0.219	0.163	0.100	0.244	0.094	0.128	0.121	0.150
K-8	0.156	0.359	-	0.100	0.147	0.175	0.152	0.238	0.267	0.128	-	0.296	0.118	0.143	-	0.152
K-9	0.054	0.200	-	-	0.044	0.191	0.317	0.205	0.107	0.177	0.091	0.286	0.147	0.143	-	0.131
K-10	0.051	0.111	-	0.273	0.091	0.103	0.326	0.214	0.281	0.056	0.057	0.333	0.147	0.171	-	0.148
Mean	0.121	0.215	0.096	0.157	0.125	0.159	0.218	0.267	0.155	0.095	0.087	0.277	0.142	0.151	0.054	0.154

Table 3. Wright's fixation indices for ten populations of *V. tetrasperm*

Pop.	*Acp-1*	*Acp-2*	*Acp-3*	*Est-1*	*Est-2*	*Got-2*	*Idh-2*	*Mdh-1*	*Mdh-3*	*Per-1*	*Per-2*	*Pgi-2*	*Skd-2*	*Sod-1*	*Sod-3*
K-1	0.687***	0.269*	0.377*	0.278	0.595***	0.716***	0.690***	0.438***	0.410*	0.638***	0.775***	0.322*	0.435*	0.367*	0.475**
K-2	0.695***	0.481***	0.188	0.718***	0.659***	0.756***	-	0.349**	0.460*	0.786***	0.845***	0.238	0.721***	0.769***	0.641***
K-3	0.619***	0.727***	0.411*	0.300	0.635***	0.731***	0.415**	0.455***	0.300	0.641***	0.722***	0.465***	0.525**	0.481***	0.788***
K-4	0.647***	0.662***	0.652***	0.208	0.584***	0.505***	0.181	0.352**	0.438*	0.468**	0.752***	0.568**	0.394*	0.515***	0.843***
K-5	0.557***	0.696***	0.484***	0.244	0.651***	0.675***	0.620***	0.402**	1.000***	0.708***	0.361*	0.685*	0.424*	0.642***	0.685***
K-6	0.372*	0.471**	-	0.188	0.718***	0.708***	0.551***	0.151	1.000***	0.725***	0.665***	0.319*	0.432*	0.712***	0.517**
K-7	0.813***	0.244	-	0.525**	0.740***	0.444**	0.388**	0.561***	0.244	0.564***	0.549***	0.170	0.526**	0.679***	0.643***
K-8	0.732***	0.386**	-	0.672***	0.614***	0.369*	0.367*	0.525***	0.267	0.678***	-	0.254	0.442**	0.617***	-
K-9	0.724***	0.502**		-	0.590***	0.787***	0.431**	0.497***	0.519**	0.402*	0.528***	0.276*	0.466**	0.492**	-
K-10	0.806***	0.606***	-	0.151	0.708***	0.528**	0.383**	0.436**	0.027	0.825***	0.770***	0.218	-0.064	0.582***	-

*p<0.05. **p < 0.01, ***p < 0.001. Monomorphic loci for a particular population is indicated with a dash.

The observed low, significant, and positive *F* value indicates that homozygotes were significantly in excess. If significant deficiencies of heterozygosity for each polymorphic locus are present, this indirectly indicates the existence of inbreeding. Generally, seedling stages are expected to have higher levels of inbreeding than found in adults [5]. These levels of inbreeding can result from a variety of causes because *V. tetrasperm* is mixed mating species; positive assortive mating (i.e., preferential mating among similar genotypes), selection for homozygotes, family structure within a restricted neighborhood, causing mating among relatives. The significant deficiency of heterozygotes found in many populations may partly be due to the fact that there has been selection favoring homozygotes among populations. This may suggest that selection against heterozygotes operated in the progeny populations throughout the life cycle. This allowed few inbred progenies to survive to the adult stage, resulting in more outcrossed adult plants. Selection in favor of heterozygotes typically occurs in more extreme environments. The reproductive strategy of *V. tetrasperm* could explain the observed inbreeding level. Because *V. tetrasperm* is autogamous species, it is expected that all of the inbreeding detected is due to consanguineous and self-mating. Nei et al. [14] have shown that the reduction in average heterozygosity per locus depends not only on the size of the population bottleneck, but also on the subsequent rate of population growth. If population growth is reduced, reduction in average heterozygosity is large, even given a small number of founder.

A substantial heterozygote deficiency in some population and at some loci. The patch distribution of related individuals should generate a Wahlund effect. The sampling included individuals from several patches per population, resulting in an overall deficiency of heterozygotes. It is probable that the combination of these factors may contribute to heterozygote deficiencies within these populations. In addition, if breeding systems and a Wahlund effect affect the population genetic structure, all *F* values for polymorphic loci should show similar patterns in a single population. *F* values were nonsignificant among polymorphic loci (Bartlett test). This is suit for *V. tetrasperma*, suggesting that the acting evolutionary forces (e.g., selection factor) similar in their impact upon polymorphic loci.

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초록 : 얼치기완두(콩과) 집단의 교배계와 내교잡 압력

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한국 내 얼치기완두(*Vicia tetrasperm*) 집단의 교배계를 알로자임 분석으로 실시하였다. 그 결과 얼치기완두는 타가수분 또는 혼합 교배 타가수분을 영위하고 있었다. 집단 수준에서 열 개 집단에 대한 내교배 계수는 0.131에서 0.176까지로 나타나며 평균은 0.154였다. 다대립좌위에서 타가수분 계수(*tm*)는 열 개 집단에 대해 0.269와 0.423 사이에 있으며 평균은 0.333이었다. 다대립좌위와 단일좌위에서 타가수분 계수 차이는 상당히 높게 나타났으며 양친과의 근친교배가 유의하게 일어나고 있었다. 일부 집단에서 낮은 타가수분율은 광범위한 근친교배와 성숙한 개체간 격리에 기인한다. 비록 한 집단에서 이형접합체 과다가 기록되었지만 대부분 집단은 이형접합체의 결핍이 관찰되었다. 따라서 동형접합체에 대한 자연선택이 생활사를 통한 지손집단에 작용하고 있었다.