Gene Flow And Genetic Diversity In Endangered Plant Population, *Morus Macroura* Miq. In West Sumatra

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ABSTRACT: In order to clarify the genetic diversity within population and gene flow between population in the endangered Andalas tree*(Morusmcraura* Miq.), RAPD analysis was conducted on 24 individuals from 3 Populations (X Koto, Tanjung Raya and Batipuh) in West Sumatra. The different of genetic diveersities were detected Among Populations of *Morus mcraura* Miq. The genetic diversity of Tanjung Raya population of *M. macroura (Na* = 2, *Ne* = 1307,*H* = 0179, *I* = 0.270) was higher than Reviews those of Batipuh population*(Na* = 1, *Ne* = 1291, *H* = 0168, *I* = 0.250) and X Koto population*(Na* = 2, Ne = 1264, *H* = 0162, *I* = 0249). Most of the total genetic diversity of *M. macroura (*H_T= 12:28) was resided within population*(*H_S = 0.225), while between population*(*$D_{ST)}$was 0.055. The genetic differentiation was slightly high*(*G_{ST} = 0.244). Total Gene flow across all Populations was slightly low*(Nm*= 1,549). There was Significantly correlated between geographic distance and genetic similarity of *M. macroura* (r = - 0.186, Mantel t-test = - 3.1305, p = 0.0009).

KEY WORDS: Endangered species, genetic diversity, gene*flow,Morus mcraura*

1. INTRODUCTION

Plant Andalas*(Morusmacroura* Miq.) Is one of the rare plants Indonesia (Mogea *et al.,* 2001). which is also the identity of the flora of West Sumatra (Rahman, 1991) and is now steadily declining number of individuals or populations can eventually become extinct due to their overexploitation but not followed by cultivation efforts. Another property that dioeceus tumbuhannya (male and female flowers are on different individuals) and phenology of reproductive isolation in the form of flowers that do not simultaneously also a factor inhibiting the formation of a fertile breeding organs (personal observation). Conservation in *situ* and *ex situ* plant was needed. But conservation programs *insitu* and *ex situ* will not succeed without the support of information and the level of genetic diversity*'geneflow'* in these populations (Hamrick *et.*Al.In Kim and Chung 1995; Frankham *et*al.,2005).

The setting of genetic diversity has become a common target in most conservation programs depicting the genetic diversity within and between natural populations. Data genetic diversity of a plant is very important because the genetic variation affects the setting of its existence in nature (Godt and Hamrick, 1996). The study of genetic variation in natural populations may al so provide important information about the evolution of a species'

genetic diversity Besides determination, determination of gene flow between populations is also necessary to look at the level of migration among this population. The average rate of gene flow between populations can be estimated from measurements of gene frequencies. According Slatkin (1977) the effects of gene flow*(geneflow)*among the local population of a species with extinction significant and the speed of re-colonization has an important role in the possibility of group selection in such species as the effectiveness of selection groups depending on their genetic differences between local populations.

Generally a very precise assessment of genetic variation performed by using isozymes as more number of genes involved in the analysis (Syamsuardi, 2002a, 2002b). But this is difficult to do at *M. macroura* because of the sampling sites away from the lab. The use of technology RAPD*(RandomAmplified Polymorphic*DNA)using dry samples will be able to overcome it. The use of RAPD technique has been performed on the assessment of genetic variation for the purpose of conservation of rare plants (Bekessy *et*al.,2002; Allphin *et*al.,1998). According Jamsari (2007) This technique is fast enough for selecting polymorphisms, as well as classified techniques that are easy and economical. For that reason this technique is widely used for studies of genetic diversity and population structure.

Trees andalas rare plants and flora mascot West Sumatra is very important to be protected. Conservation efforts would be meaningless if it is not supported by the data of genetic diversity within populations and *gene flow* between populations. Due to the continuation of the kind of life that depends on how plants regulate*(maintain)*its genetic diversity. Based on the explanations above, the study of genetic variation in the population and the level of *gene flow* between populations of plants andalas is very necessary.

2. RESEARCH METHODOLOGY

24 samples of three populations *of M. macroura* in West Sumatra, namely X Koto (6 people), Batipuh (8 people) and Tanjung Raya (10 people). Fresh samples from young leaves are put in plastic containing silica gel was then taken to the lab to do DNA isolation ekstrkasi to do. DNA was isolated using the CTAB method (Hilis and Moritz, 1990). Five primer (OPA-16, OPA-17, OPA-18 SBH 13 and SBH-19) has been chosen because it has a high polymorphism and consistency of the ribbon that appears. RAPD is a dominant marker so that between homozygous and heterozygous individuals are not distinguishable. Scoring against RAPD data is performed based on the presence or absence of bands*(band).*Samples have given a score of 1 while the tape that does not have the tape was given a score of 0. The parameters such as the percentage of polymorphic loci (P), Nei genetic diversity*(H),*phenotypic diversity index Shannon*(I),*heterozygosity in the population*(H_S)*and total heterozygosity*(H_T),*coefficient of genetic differentiation*(G_{ST})*and *gene flow (Nm)*was analyzed by using the program POPGENE 1:20 (Yeh *et*al.,1997). Value *gene flow* is calculated using the formula $Nm = 0.5 (1-G_{st} / G_{st})$.If *Nm* more than 1, *gene flow* will neutralize *genetic drift* within the population and if *Nm* is smaller than 1, then the *genetic drift* is a major factor shaping the population structure (Levin, 2000). 2:02 NTSYSpc program (Rohlf, 1998) is used for clustering analysis based on genetic similarity coefficient data *Jaccard* and morphology as well as the determination of genetic distance relationship with the morphology of leaves and the geographical distance by *Mantel*test.

3. RESULTS AND DISCUSSION

Six primers used in this study yielded 40 tape size range of 300 bp - 6000 bp. Of the forty-tape generated, tape 30 polymorphic and 10 monomorphic banding pattern that shows the same in all individuals tested. Primer OPA-16 resulted in the number of bands the most (9 band) followed by the OPA-18 (8 pita), OPA-17 and SBH-7 (7 tape), SBH-19 (6 pita) and the least was OPA- 14. In Figure 2 visualized band pattern polymorphic and monomorphic.

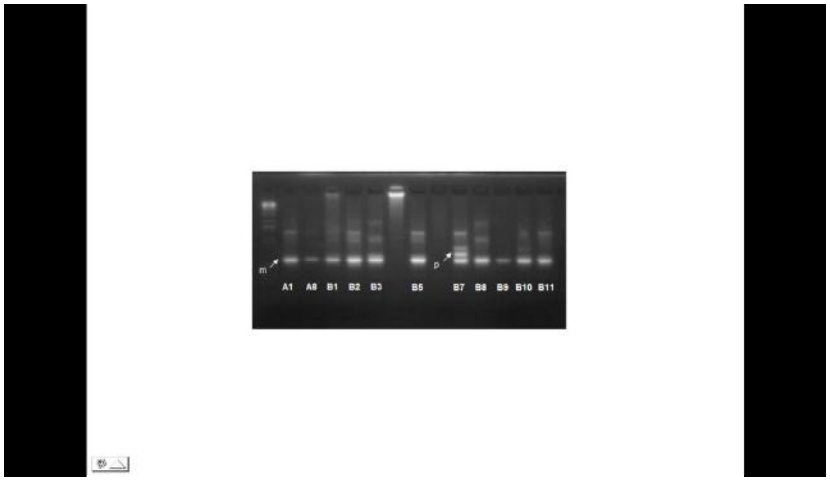

Figure 1. Visualization electrophoresis DNA sample after PCR amplification. X Koto population sample (A1), Batipuh (A8) and Tanjung Raya (B) visible presence polymorphic bands (p) and monomorphic (m).

Table 1. Total ribbon, monomorphic and polymorphic resulting from amplification of DNA in 26 people using 6 RAPD

PitaPolymorphic	Pitamonomorphic	Total Pita	SequenceBases (5 '→ 3')	Primers	No.
3	0	3	TCTGTGCTGG	OPA - 14	1
7	2	9	AGCCAGCGAA	OPA - 16	2
6	1	7	GACCGCTTGT	OPA - 17	3
5	3	8	AGGTGACCGT	OPA - 18	4
4	3	7	CACACCGCAC	SBH - 13	5
5	1	6	CACCCACGAC	SBH - 19	6
30	10	40		Total	

the results of the analysis of the profile of each tape 3 individuals from the population, there is a tendency looks different banding pattern among three populations studied and found unique bands contained in a population and not found in other populations. In Figure 2 are shown in a unique position on the third tapepopulation. *M. macraura* Individuals at Batipuh area as much as 87.5% of them had a blue ribbon and in the area of X Koto contained in four individuals (66.7%), while the band is only found in one individual (10%) in Tanjung Raya. Individual area of Tanjung Raya 50% of them had a pink ribbon. The fact it could demonstrate that people in the area Batipuh and X Koto rich in certain genes (as indicated by the size of 3000 bp band profile on OPA-18) that these genes are rarely found in the area of Tanjung Raya. Similarly, the individual area of Tanjung Raya generally rich in certain genes (depicted by the band profile sized 1700 bp at the SBH-19). From Figure 2 above, it is assumed there is genetic differentiation between populations of plants andalas Batipuh, X Koto and population Tanjung Raya. This assumption is evidenced by the results of the analysis of grouping the next discussion.

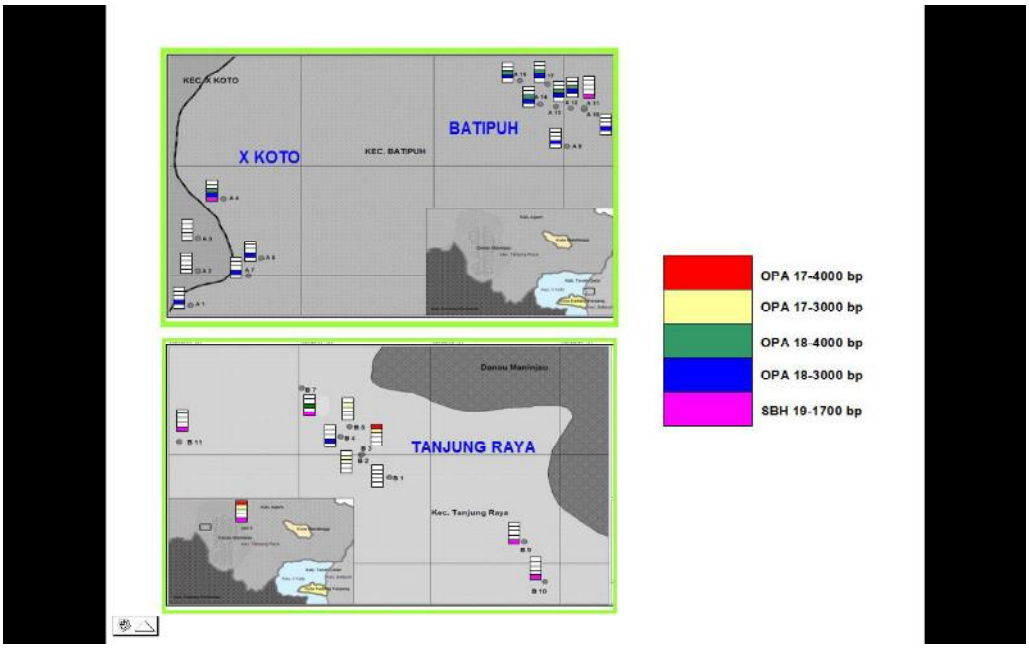

Figure 2. The pattern of some unique ribbon contained in a population of X Koto, Batipuh and Tanjung Raya.

Statistical Analysis Resultsgenetic diversity *M. macroura* in each population (Batipuh, X Koto and Tanjung Raya) showed that the genetic diversity in the population X Koto, Batipuh and Tanjung Raya is quite high. Values obtained the highest genetic diversity in populations Tanjung Raya with an average value of heterozygosity *(H)*of 0179 and the average value of Shannon index*(I)*of 0270, and then in the population Batipuh value *H* of 0168 andvalue *the first* of 0.250. Population X Koto has a value *H* that is equal to the lowest0162 and *I* of 0249 (Table 2).

Table 2. M. macroura genetic diversity in populations X Koto, Batipuh and Tanjung Raya.

I	*H*	*Ne*	*Na*	The number of samples	Population
0249 ± 0271	0162 ± 0185	1264 ± 0331	2	6	X Koto
0.250 ± 0.291	0168 ± 0.204	1291 ± 0378	1	8	Batipuh
0270 ± 0287	0179 ± 0202	1307 ± 0374	2	10	Tanjung Raya

Description: Na: Average -rata number of alleles observed; Na: the average number of alleles; Ne: The average number of effective alleles; *H:*Average heterozygosity; *I:*Average Index Shannon (Lewontin, 1972)

The difference in the value of heterozygosity in the population X Koto, Batipuh and Tanjung Raya assumed, among others, related to the size of the population (number of people). In ideal conditions, the size of the population with larger populations have greater heterozygosity compared with smaller populations. This is in accordance with the opinion of Frankham *et*al.(2002) which states that the reduction in population size*(bottleneck)*is the main thing that caused the loss of genetic diversity, in particular due to thesize

effective populationis usually smaller than the number of reproductive individuals in the population.

An effective population size of 100 (size for the type of *vulnerable*species)lost 25% value heterozigositasnya for 57 generations. Hetero-zygosity reduction is dependent upon the generation that has the effective population size is the smallest. As an example of an effective population size of 10, 100, 1000, and 10,000 for four generations lost 5.5% value heterozigositasnya (almost equal in value to the loss of a generation on the population size of 10). Rare species such as *M. macroura* has a relatively small population size, so this plant is likely to have a lower genetic diversity compared with species that are not endangered*(non-endangered*species).

The result of the calculation of the total value of heterozygosity (H_T)in a population of 0225 can be seen that a very large genetic variation in *M. macroura* overall (population X Koto, Batipuh and Tanjung Raya). Values in the population heterozygosity$(H_S = 0.170)$ greater than the value of heterozygosity among populations$(D_{ST} = 0.055)$.

Table 3. Distribution of genetic diversity, genetic differentiation and gene flow among populations of M. macroura

N_m	G_{ST}	D_{ST}	H_S	H_T	number of samples
24					0,225 0,170 0,055 0,244 1,549

The high value of H_S (0.170) compared to the value D_{ST} (0055) showed thatgenetic variation *M. macroura* in the population is higher than the genetic variation among populations. This may be related to the reproductive biology of *M. macroura* that *out-crossing,*because the individual male and female at the *M. macroura* is separate*(dioceous).*Unknown plant pollination is helped by the wind (anemogami). Reproductive biology of plants with *out-crossing* gives a greater opportunity to increase the value of heterozygosity in the population. Hamrick and Godt (1996) also stated that in general *selfing species* usually have a low genetic diversity in the population and higher genetic differentiation among populations relative to *out-crossing*species.

The analysis showed that the value of genetic differentiation*(G* ST) between populations X Koto, Batipuh and Tanjung Raya of *M. macroura* is 0.244 with the value of its gene flow*(Nm* = 1.5). Value *Nm>* 1 indicates that the levels of gene flow between populations is able to cope with genetic drift. Value *Nm* <1 indicates a genetic drift as a cause of population differentiation (Slatkin, 1987). The decline in the population size is one of the factors hamper the flow between populations triggered by habitat fragmentation and exploitation of this plant. Habitat fragmentation often cause an increase risk of extinction that also happens to *M. macroura* as flora mascot of West Sumatra.

Analysis of the grouping of the 24 people on three populations of M. macraura basedsimilarity index *Jaccard* produce fenogram as shown in Figure 3. All individuals grouped based on the origin of the population despite the exceptions in individual B7 (Tanjung Raya) joins a group of individuals A11 (Batipuh) , This fact indicates that the population structure has been formed in this plant as a result of genetic differentiation between all three populations. B7 separate individuals with individuals in the population Tanjung Raya other and enter into Batipuh population groups. It can be assumed that the individual may have originally come from populations Batipuh or X Koto due to the distribution center of the individuals who have *gene pool* the sameis in Batipuh or X Koto. Another possibility is due to the pattern dispersalnya aided by birds or locals.

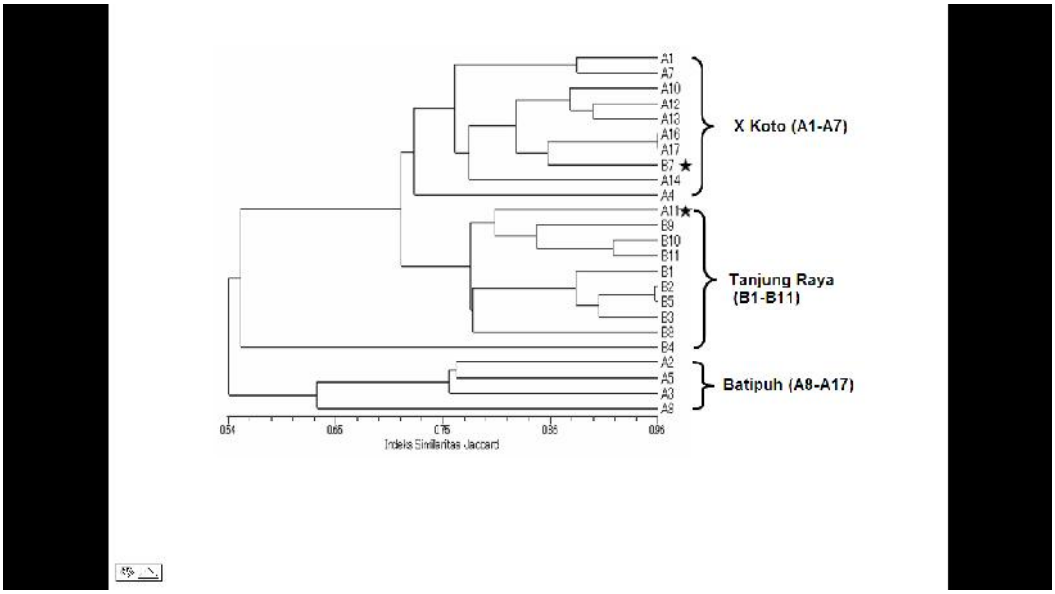

Figure 3. Fenogram cluster analysis (UPGMA) RAPD of data on individuals M. macroura of three populations (X Koto, Batipuh and Tanjung Raya)

Furthermore, the population structure is formed (Figure 3) associated with the geographic distance between populations. For a proof it has carried out the analysis of correlation between genetic distance and geographical distance which showed a significant correlation between genetic differentiation and geographic distance between populations (r = -0186, Mantel t-test = -3.1305, p = 0.0009) (Figure 6). This indicates that geographic isolation plays an important role in the formation of the genetic differences in populations *of M.*macroura.Syamsuardi research results (2002b) showed that differences in the genetic structure because of the gap also occurs in plants *Ranunculus japonicus* in Japan.

In general, the study of genetic diversity, genetic differentiation or population structure often associated with morphologic variation due to theevents, *rapid speciation* genetic differentiation will be followed by the morphological differentiation (Stuessy, 1990). Correlation analysis similarity of morphological characters with genetic similarity with Mantel test showed that there is no relationship between genetic variation in morphological variation (r = 0.025, t-test Mantel t = 0604, p = 0.2587) were significant morphological differentiation is not accompanied by genetic differentiation. Analysis grouping with PCA is based on the similarity of morphological characters also showed the group formed is not referring to the population group.

4. CONCLUSION

From the research that has been done, it can take some conclusions as follows:

1. Population *M. macroura* in Tanjung Raya has a higher genetic diversity (Na = 2, Ne = 1307, *H* = 0179, *I* = 0.270) compared with the population in Batipuh (Na = 1, Ne = 1291, *H* = 0168, *I* = 0.250) and X Koto (Na = 2, Ne = 1264, *H* = 0162, *I* = 0249).
2. Ratedheterozygosity *M. macroura* higherin the population*(H_S* = 0.225) than heterozygosity among populations*(D_{ST}* = 0.055). Genetic differentiation between populations was high*(G_{ST}* = 0.244) with a value of gene flow*(Nm)*at 1,549.
3. Genetic differentiation in *M. macroura* associated with geographic distance (r = - 0.186, Mantel t-test = - 3.1305, p = 0.0009).genetic differentiation *M. macroura* is not related to the morphology of leaves (r = 0.025, t-test Mantel t = 0604 and p = 0.2587)

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