Control Mechanism of Branch Formation of Kenaf (Hibiscus Cannabinus L.): Genetic Approach

Estri Laras, Arumingtyas, Retno.Mastuti, Serafinah.Indriyani

Abstract— The aim of this research was to determine the mechanism of branching formation on kenaf. True lines basal branching plant was crossed with apical branching plant. The first filial (F1) was observed for its segregation. The first filial (F1) of the crossing between non branching control plant and basal or apical branching plants consist of almost 100% branching, regardless their basal or apical branching parent, showing that both basal branching and apical branching were controlled by recessive alleles. The F1 of crossing between basal and apical branching plants indicates the existence two genes that control both type of branching. Identification of branching gene using PCR technique indicate that branching gene of kenaf was homologous to auxin signalling branching gene of Arabidopsis thaliana. It was suggested for different type of branching was controlled by different allele of the same locus.

Keywords—branching gene, kenaf, auxin, mutation

I. INTRODUCTION

The existence of several genes controlling shoot branching formation of several plant species has been reported. Mutants that specifically lost its ability to control the development of lateral meristem give the opportunity for investigating genes involved in branching formation. In kenaf, mutation induced by Ethyl Methane Dsulfonate (EMS) cause the changing in the sequence of AUX1 gene [1], [2]. In Arabidopsis, supershoot/bushy mutan is controlled by the cytochrome P450 gene [3], [4]. The gene responsible for the formation of teosinte branched1 (tb1) branching type on maize possibly act as regulator of the expression of transcription which is expressed in the shoot branching [5] and act to suppress the growth and determination of branches. The emergence of branches on plants also has been known to have association with the phenomenon of apical dominance. While apical dominance believed to be associated with the presence of auxin although the mechanism of how auxin affects apical dominance is still a question. So far auxin proved to directly control the formation of branches [6], [7], [8]. There are indications of the role of cytokines in conjunction with the effect of auxin in the appearance of branches [9]. However until recently the mechanisms controlling branching by auxin and cytokinin and the linkages between these two hormones is unclear.

Various studies have pointed to the existence of a signal, or the substance that connects auxin level by the number of branches, but never identified the type of substance. Seeing the lack of identification of the substance, as well as the emergence of auxin when branching always appear, perhaps even balance between auxin and cytokinin levels which play a role in controlling the formation of branches hormones [10]-[15].

Mutation in three loci of DAD (Decreased Apical Dominance) in petunia and five loci of RMS (Ramosus) in pea [16] cause the elimination of branching inhibition. Those genes decrease the inhibition of branching formation and show pleiotrophic effect to the characters which has no relation to the branching [17]. Cloning of MAX2 gene showed the indication of ubiquitin mediated protein degradation role in the inhibition of branching formation. Moreover, the existence of AXR1 and its ortholog, AUXI from Arabidopsis has been confirmed to be an important part for auxin resposes. Profusely branching phenotype showed by mutant plant axr1 has been utilized as a genetic evidence for the role of auxin in the branching formation [18].

II. MATERIAL AND METHODS

A. Crossing Experiment

CROSSING EXPERIMENT was conducted between true lines of each branching mutant with their initial line (KR 11), and between true lines of basal branching and apical branching mutants. The first filial (F1) of those crossing were observed for determine the segregation pattern.

B. Molecular Identification

DNA Isolation and Polymerase Chain Reaction (PCR)

DNA genome of kenaf was isolated from the leaves using the CTAB methods [19], PCR was conducted using 5 pairs primer consisted of 4 pairs of specific primer designed from the sequences of AUX1, AXR1 of Arabidopsis thaliana, RMS1 of Pisum sativum, and Ls from Lycopersicum esculentum, and one degenerate primer pair designed based on the amino acid conserve of LAS, Ls and Moc. Each primer pair were mixed
with 2 ul of 10X buffer Taq Polymerase, 1.6 ul of 200 μM
dNTP, 1.6 μl of 2mM MgCl2 and 1U  Taq Polymerase.

The condition used was 35 cycles of PCR reaction
consisting of 1 minute denaturation at the temperature of 93°C,
30 seconds annealing at the temperature of 56°C, and 1 minute
extension at the temperature of 72°C. A preheating period was
applied for 1 minute at the temperature of 93°C, and the last
eelongation step was conducted at 72°C for 10 minutes. The
PCR result then was run on an electrophoresis on  1.5 %
agarose gel with  TBE buffer.

III. RESULT AND DISCUSSION

A. Determination of the number and the character of
gene controlling branching habit

Observation of branching character of the first filial
of crossing between basal branching and apical branching mut-
ants and the initial line, resulted in some phenomenon describe
below (Table1).

<table>
<thead>
<tr>
<th>Crossing</th>
<th>Filial 1</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>B</td>
</tr>
<tr>
<td>Control X Basal</td>
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<td>0</td>
</tr>
<tr>
<td>Control X Apical</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Basal X Apical</td>
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<td>0</td>
</tr>
<tr>
<td>Control Apical</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Apical</td>
<td>0</td>
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</tbody>
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The control initial line plant (KR11) show some retarded
branches with the length of less than 10 cm (Figure 1 A). This
phenotype is specific for KR11. These retarded branches will
not develop into true branches and do not produce flower, and
generally, when the plant already entering generative phase the
retarded branches do not grow longer. Different from the
branching type plants which capable to initiate and produce
branches which are eventually capable to produce flower and
after the plant have entered generative phase the development
of branches were enhanced. Basal branching or apical
branching plant showed long branches which eventually
produce flower and capsule (Figure 1B).

Crossing experiment between control and basal branching
mutant resulted in 100 % non branching F1. The phenotype
of the F1 plant was similar with the control in having retarded
branches which were not grow into true branches when the
plant entering generative phase. This indicated that alelle
controlling basal branching was recessively inherited which
was not expressed when the plant was crossed with the non
branching initial line which possess dominant allele for the
gene.

Similar result was found in the cross between non branching
control with apical branching mutant. The first filial consisted of
90.48 % (19 plants out of 21 plants) non branching plants
and 9.5 % (2 plants from 21 plants) grew apical branches.
Similar to the result of crossing between basal branching
mutant with the initial line, this non branching  F1 also has
similar phenotype to the initial line. This phenomenon also
indicate that the apical branching character was controlled by
recessive allele which is not expressed when the dominant
allele was exist.

Based on the those two crossing experiment it was
indicated that the two type of branching behaviour were
controlled by recessive allele. However, whether those two
alleles were similar, was not known yet. To confirm those
possibility were test with the cross between basal branching
with apical branching mutants. This cross resulted in the F1
consisted of 46.67 % non branching plant, 46.67 % apical
branching plant and 6.67 % showed basal-apical branching
appearance. The non branching F1 plants show similar
phenotype with the initial line KR11, on the other hand the
apical branching F1 has similar phenotype with the apical
branching parent. The evidence of non branching F1 aroused
from crossing of two branching type mutant indicate the
existence of two loci in controlling those two types of mutants.
The first locus which control apical branching were
homozygote recessive (aa) when it is control the apical
branching mutant and heterozygote (Aa) in the basal branching
mutant. The second locus was homozygote recessive (bb) in
the basal branching plant, and homozygote dominant (BB) in
the apical branching plant. At the F1, the recessive allele of
locus A from the apical plant were paired with dominant allele
of locus A of non branching plant resulted in 50 % non
branching plants.

The evidence of two different loci which controlled the two
type of branching seem to be in line with the proposed
mechanism of branching formation which regards to the
existence of growth hormones auxin and cytokinine and the
ratio of both hormones [13], [15]. Auxin produced in the
apical shoot and transported basipetally, whereas cytokinine
was produced in the root and transported to any direction [11].
Auxin causes the inhibition of branching formation [10], [12],
[14]. To deliver the inhibition effect to the branch meristem,
signal was needed, and eventually a proper ratio of auxin-
cytokynine is also needed. The time of emergence of basal and
apical branching were almost similar with the basal branches
were emerge slightly quicker. It seem that the initiation of
basal branches and apical branches were happen in the same time but were controlled by different allele.

The existence of auxin in the apical shoot could be related to the formation of apical branching, and the existence of cytokinine in the root could be related to basal branching formation. However this need further confirmation. More confirmation also need to be done to ascertain F1 self pollination produced 9:3:3:1 F2 segregation to confirm the evidence of two gene control branching formation.

B. Molecular Identification of Branching Gene

From five primer used for amplification, only AXR1 and AUX1 which has been capable of amplifying DNA genome of control, apical branching, and basal branching plants and the F1 of crossing between those plants (Figure 2).

![Figure 2. PCR result of genome DNA using primers RMS1 (a), AUX1 (b), AXR1 (c), Ls (d) and Lim (e). Show the amplification band](image)

The size of band resulted from the amplification were similar, which indicate similar molecular weight. However similar molecular weight does always reveal similar DNA sequence. The genes AXR1 and AUX1 are responsible in convey the auxin signalling at after the initiation of branches. AXR has been identified as the member of gene family AUX/IAA. So, the similarity in size between AXR1 and AUX1 PCR result showed that AXR1 and AUX1 are homologous [14], [18], [20], [21].

From four genes used as the base of specific primer design Ls is the earliest gene that has a role in the process of branching formation. Ls responsible in the GA signalling which may related in the branching initiation and breaking bud dormancy because of the synthesis of ABA [22]. The next performing gene is RMS1 which has a role in transport auxin signal in the process of breaking apical dominance [23], [24]. In turn the AXR1 and AUX1 genes act in the latest phase, after the branch emerging, to determine whether the branches continue to developed or ceased growth [18]. This sequence of gene action matches to the morphology observation of kenaf plant. The control plant and the non branching F1 plant always produce retarded branches (Figure 2). This indicate that initiation of the branches has been done and bud dormancy has been broken, however for the plant that has dominant allele for AUX1 doesn’t continue develop branches, on the other hand the recessive allele (aux1) cause the development of branches continue.

IV. CONCLUSION

Basal branching and apical branching was controlled by different gene. Those genes in the form of recessive allele cause the formation of branches. Molecular analysis indicated the existence of genes responsible in auxin signalling, although different type of branches was controlled by different allele. The gene was act in the last phase of branching formation control, by controlling auxin signalling to determine the continuity of branches development.

Further research is needed to confirm the existence of the two genes in controlling the two types of branching habit. Also the existence and concentration and ratio of auxin and cytokinin hormones need to be measured to detect the role of those hormones in the branching formation.

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