SCAR (Sequence Characterized Amplified Region) Analysis for Blast Resistant Evaluation on 12 Genotypes of Rice

Sobir¹, Harmi Andrianjyta¹, Mukelar Amir²

ABSTRACT

Resistance evaluation to blast disease (Pyricularia grisea) on 12 paddy genotypes was carried out in the green house by using spray inoculated method with race 033 and 041 of P. grisea, and SCAR (Sequence Characterized Amplified Region) marker by using Pib primer pairs. The results revealed that among 12 paddy genotypes were classified into six resistance groups. The first group comprised two genotypes (Jatiluhur and Asahan) having three resistance genes. The second group comprised two genotypes (Oryza malampuzhensis and O. punctata) having two resistance genes against race 033 and 041. The third group had one resistance gene against race 033, comprised one genotype (Way Rarem). The fourth group comprised one genotype (Danau Tempe) having two resistance genes against 041 race and Pib. The fifth group comprised three genotypes (Kalimutu, Maninjau and Laut Tawar) having two resistance genes against race 033 and Pib. The sixth group comprised three genotypes (Kencana Bali and Cirata) having no resistance gene to blast race 033 and 041, and Pib. These results indicated that Pib gene did not confer resistance to race 033 and 041 of Pyricularia grisea. Resistance to race 033 and 041 might be controlled by different resistant gene.

Key words: SCAR, Blast resistant, Rice

INTRODUCTION

Rice blast, caused by the fungal pathogen Pyricularia grisea, is the most serious disease for upland. However, recently it has been reported that the pathogen also infest irrigated rice (Amir et al., 2000). The fungus attacks leaves during early growth stages, develops lesions that are followed by premature leaf senescence of infected tissues, especially in case of heavy infections. After heading, the pathogen infects the panicles or the neck, giving high lost of yield. The use of resistant cultivars is the most effective means on controlling the diseases, however, the useful life span of many cultivars is only few years, due to breakdown of the resistance in the face of high pathogen variability of the fungus (Kiyosawa, 1982).

The genes conferring resistance to rice blast has been studied extensively. So far at least 30 resistance loci have been identified in rice (Inukai et al., 1994), and several loci have recently been mapped by using Restriction Fragments Length Polymorphism (RFLP) markers (Yu et al., 1996; Nakamura et al., 1997). Wang et al. (1999) was successfully isolated and characterized Pib gene, one of the genes conferring resistance to rice blast disease, by using map-based cloning strategy. The availability of information regarding the complete sequence of Pib gene leads to the possibility of developing specific primers to mark the Pi-b gene. These markers are classified as Sequence Characterized Amplification Region (SCAR) markers, which offer advantage on accuracy over RAPD markers, since the primer consist of more than 20 bases, and simplicity over RFLP markers. Detection of SCAR markers does not need laborious steps of blotting, hybridization and detection (Sobir, 2000).

Resistance to blast diseases in rice is conferred by R-genes that named as Pi genes (Ou, 1985). The Pi genes act as major gene, which recognize specific rice blast race, following gene-for-gene hypothesis (Ebron et al., 2002). To date 25 Pi genes have been identified already (Fukuta et al., 2002), located in several loci on rice genome (Wang et al., 1999). To date, based on reactions pattern to 7 differential varieties, in Indonesia have been identified 27 races of P. grisea (Amir et al., 2000), but was not available information, wheather resistance to each of these races controlled by specific
Pi gene or not. The dominant Pi-b gene is confers high resistance to most Japanese blast race, but in Indonesia, is not well identified yet, particularly in what fungus race that the gene to be confers and what varieties that carrying the gene.

MATERIALS AND METHODS

Plant and Inoculum Materials

Eleven rice genotypes, consisting of two wild species of (1) Oryza malampuzhaensis, (2) Oryza punctata and nine cultivated varieties of (3) Jatiluhur, (4) Cirata, (5) Way Rarem, (6) Laut Tawar, (7) Marinjau, (8) Danau Tempe, (9) Kalimutu, (10) Asahan (as control of resistant genotype) and (11) Kencana Bali (as control of susceptible genotype) were examined.

Two races of fungus, race 033 and 041, were used in this experiment, since both of them widely found in paddy field in Indonesia (Amir et al., 2000). Inoculation materials were developed from fresh isolated conidia from the leaf, which infected by P. grisea race 033 and 041. They were cultured in PDA (Potato Dextrose Agar) media for 5 days, subsequently transferred to OMA (Oat Meal Agar) media and cultured for 10 days.

Blast Infection Assays

All evaluated genotypes were planted in a culture box containing clay soil 6 days after germination in greenhouse. Inoculation was conducted to the rice leaf 18 days after planting, by using compressor connected glass atomizer; each box was sprayed with 50 ml fungus spore, containing $3 \times 10^6$ spore/ml. After inoculation the plants were placed in humid room for 24 hours, and then transferred into greenhouse for observation.

Observation of diseases infection intensity was conducted 5 and 9 days after inoculation based on IRRI criteria, and obtained data were analyzed by following equation (IRRI, 1996).

$$Z = \frac{\sum n_i v_i}{N V}$$

Where: $Z =$ infection intensity
$n_i =$ plant number-i
$v_i =$ score of plant number-i
$N =$ number of observed plant
$V =$ maximum score base on IRRI criteria

SCAR Analysis

DNA sample of the 12 evaluated genotypes were extracted from 1 g young leaves of 6 days rice seedling by using CTAB extraction method (Doyle and Doyle, 1987) with slight modification (Sobir, 2000). Quantity and quality of extracted DNA was examined by electrophoresis method.

SCAR analysis was conducted by amplification DNA samples of 12 genotypes of rice by using pair of 20 mer Pi-b primer designed from mRNA sequence of Pi-b gene (Wang et al., 1999). The primers sequence are 5'-AGGGAAAAAT GGAAATGTGC-3' (sense) and 5'-AG TAACCTTCTGTGCCCAA-3' (anti-sense). Polymerase Chain Reaction (PCR) was performed in 25 ml reaction containing of 2.5μL of 10X buffer, 1.5μL of 25 mM MgCl₂, 1 μL of 2.5 mM dNTPs, 1 μL of 10 pM of each primers, 1 μL of 100 ng DNA template and 1 unit of Taq DNA polymerase enzyme. Amplification was carried out by using Perkin Elmer 9700 PCR machine under following conditions Pre-PCR at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute. PCR was stopped at 72°C for 7 minutes. PCR products were resolved in 1.0% of agarose gel.

RESULT

Observation was conducted on the susceptible type spot, as indicated by gray color on the center of the spot, since this spot is the source of conidia for secondary infection. Based on spot observation at 5 days after inoculation (dai) and 9 dai, infection intensity data were presented in Table 1. Infection intensity score below 25 indicates that the genotype is resistant and higher than 25 indicates that the genotype is susceptible. Based on the infection intensity score criteria, it was found that Danau Tempe, Cirata and Kencana Bali genotypes were susceptible to Pyricularia grisea race 033, and Laut Tawar, Marinjau, Kalimutu, Cirata and Kencana Bali genotypes were susceptible to Pyricularia grisea race 041.

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Table 1. Infection intensity on 5 days after inoculation (dai) and 9 dai of the fungus race of 033 and 041 on 11 rice genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Race 033</th>
<th></th>
<th>Race 041</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 dai</td>
<td>9 dai</td>
<td>5 dai</td>
<td>9 dai</td>
</tr>
<tr>
<td><em>Oryza malampuzhaensis</em></td>
<td>2.5</td>
<td>0.0</td>
<td>1.6</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Oryza punctata</em></td>
<td>12.3</td>
<td>0.0</td>
<td>4.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Jatiluhur</td>
<td>10.2</td>
<td>6.70</td>
<td>10.8</td>
<td>14.2</td>
</tr>
<tr>
<td>Asahan</td>
<td>0.0</td>
<td>0.0</td>
<td>3.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Laut Tawar</td>
<td>22.7</td>
<td>20.2</td>
<td>30.3</td>
<td>38.0</td>
</tr>
<tr>
<td>Maninjau</td>
<td>11.7</td>
<td>10.3</td>
<td>43.0</td>
<td>43.6</td>
</tr>
<tr>
<td>Kalimutu</td>
<td>27.2</td>
<td>20.9</td>
<td>39.9</td>
<td>29.0</td>
</tr>
<tr>
<td>Way Rarem</td>
<td>3.7</td>
<td>10.0</td>
<td>27.7</td>
<td>24.0</td>
</tr>
<tr>
<td>Danau Tempe</td>
<td>18.5</td>
<td>33.9</td>
<td>11.3</td>
<td>9.9</td>
</tr>
<tr>
<td>Cirata</td>
<td>31.1</td>
<td>31.5</td>
<td>26.9</td>
<td>33.2</td>
</tr>
<tr>
<td>Kencana Bali</td>
<td>90.5</td>
<td>58.6</td>
<td>76.0</td>
<td>78.0</td>
</tr>
</tbody>
</table>

dai (day after inoculation)

Examination of *Pib* existence in the genome of evaluated genotypes was detected with single band of 730 base pairs of amplification product *Pib* SCAR primer. The *Pib* SCAR marker analysis revealed that Jatiluhur, Maninjau, Kalimutu, Way Rarem, Danau Tempe and Cirata genotypes carried *Pib* gene (Figure 1). Correlation analysis showed that the existence of *Pib* gene is not corresponding to the resistance responses of the evaluated genotypes, neither to race 033 or race 041.

Figure 1. SCAR marker analysis by using *Pib* primer pair. Lane no 1 to no 11 represent *Oryza malampuzhaensis*, *Oryza punctata*, Jatiluhur, Asahan, Laut Tawar, Maninjau, Kalimutu, Way Rarem, Danau Tempe, Cirata, and Kencana Bali, respectively. M is DNA size markers (λ DNA/Hind-III digest).

Based on resistance responses to race 033 and race 041, and existence of *Pib* gene in the genome, the evaluated genotypes can be classified into six groups. First group consisted of Jatiluhur and Asahan, which were resistant to race 033 and 041, and carried *Pib* gene. Second group consisted of *Oryza malampuzhaensis* and *Oryza punctata*, which were resistant to race 033 and race 041, but did not carry *Pib* gene. Third group consisted of Kalimutu, Maninjau and Laut Tawar, which were resistant to race 033 and carried *Pib* gene. Fourth group consisted of Danau Tempe, which were resistant to race 041 and carrying *Pib* gene. Fifth group consisted of Way Rarem, which was resistant to race 033 only. Sixth group consisted of Cirata and Kencana Bali, which were susceptible to race 033 and 041, did not carry *Pib* gene (Table 2).
Table 2. Classifications of rice genotypes base on resistance respond to \textit{P. grisea} race 033 and race 041, and existence \textit{Pib} gene in the genome

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Race-033</th>
<th>Race-041</th>
<th>Pib-SCAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Jatiluhur</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Asahan</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td>\textit{Oryza malampuzhaensis}</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>\textit{Oryza punctata}</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Kalimutu</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Maninjau</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Laut Tawar</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>Danau Tempe</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>Way Rarem</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>Cirata</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Kencana Bali</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

To date 25 \textit{Pi} genes have been identified (Fukuta \textit{et al.}, 2002), located in several loci on rice genome (Wang \textit{et al.}, 1999). The \textit{Pi} genes act as major gene, which recognize specific blast race, following gene-for-gene hypothesis to induce defense mechanism against blast fungus (Ebron \textit{et al.}, 2002). Therefore differences of resistance among rice varieties to different races might due to different type of \textit{Pi} genes that carried by certain rice varieties. Subsequently, differences of resistance response among rice genotypes to \textit{P. grisea} race 033 and 044 (Table 2), was probably due to resistance to race 033 conferred by different \textit{Pi} gene that confers resistance to race 041. In other hand, since the existence of \textit{Pib} gene was not associated to resistance response to both races (Table 2), this indicated that \textit{Pib} gene did not confer resistance to \textit{P. grisea} race 033 and 044.

SCAR marker analysis result showed that the \textit{Pib} gene did not exists in the genome of \textit{Oryza malampuzhaensis} and \textit{Oryza punctata}, indicating that \textit{Pib} gene was not originated from both wild species. According to Ebron \textit{et al.} (2002), \textit{Pib} gene is already carried by several \textit{Oryza sativa} var. indica genotypes such as Indonesian indigenous Peta variety. In other experiment we found that \textit{Pib} gene is carried by wild species \textit{Oryza rufipogon} (unpublished data), thus raise possibility that \textit{Pib} gene is originated from \textit{Oryza rufipogon}.

Wide range availability of \textit{Pi} genes in rice gene pool in conferring resistance to several races of blast fungus \textit{Pyricularia grisea} (Ebron \textit{et al.}, 2002) raise the possibility to develop rice varieties with field resistance through pyramiding of several \textit{Pib} gene into one genotype, as has been exhibited by Jatiluhur and Asahan.

REFERENCES


Fluoresen Klorofil Benih: Parameter Baru dalam Penentuan Mutu Benih

Seed Chlorophyl Fluorescence: A New Parameter in Quality Seed Testing

Mohamad Rahmad Suhartanto

ABSTRACT

It has been shown that chlorophyll content of seeds was negatively correlated with germinability towards the end of maturation. Physiological maturity was achieved when the chlorophyll fluorescence reached a minimum. This equipment is able to measure and analyze chlorophyll fluorescence in the seed instantaneously and non-destructively. The use of LIF makes it possible to perform physiological and biochemical assays after chlorophyll fluorescence measurement in the same seeds. Based on the results from some experiments as well as the literature, the role of chlorophyll in developing seeds is presented. The overall conclusion is that chlorophyll is required during seed development, but undesirable during maturation. We hypothesize that the presence of chlorophyll during seed maturation is undesirable since it is associated with lower quality, particularly lower seed longevity. Chlorophyll may also be a primary source of free radicals. Seed chlorophyll fluorescence was affected by endogenous abscisic acid, gibberellins and phytochrome. Light, temperature and relative humidity may also influence the chlorophyll fluorescence of seeds.

Key words: Chlorophyl, Fluorescence, Parameter, Seed testing
Bisa dibedakan dengan mata), alat pengukur dan pemilah benih berdasarkan fluoresen klorofil ini mampu memilah benih yang memiliki perbedaan warna (hijau/klorofil) yang sangat kecil yang tidak mampu diamati dengan mata telanjang, seperti pada benih tomat, cabe, kubis, wortel dan lain-lain. Benih-benih ini sangat sulit dipisahkan karena pada periode pemasakan memiliki ukuran, bentuk dan berat yang relatif sama. Karena relatif merupakan parameter baru, fluoresen dari klorofil benih diharapkan dapat bersinergi dengan parameter fisiologis lainnya untuk mengungkap masalah mutu benih.

**Fluoresen Klorofil sebagai Penciri Mutu Benih: Kasus Benih Tomat**

Kandungan klorofil pada benih tomat berkorelasi negatif dengan daya berkecambahnya (Gambar 1). Masak fisiologis yang dicerminkan oleh daya berkecambah mencapai maksimum pada saat kandungan klorofil mencapai minimum. Mutu benih sangat ditentukan oleh tingkat kemasakan benih tersebut. sehingga dapat dikatakan juga bahwa kandungan klorofil benih juga menentukan mutu benih tersebut.

**Gambar 1.** Maksimum daya berkecambah dan berat kering benih terjadi saat fluoresen dari klorofil benih mencapai minimum (Suhartanto, 2002).

Dengan menggunakan LIF tingkat kemasakan benih tersebut dengan mudah dapat ditentukan, karena fluoresen benih tomat berkorelasi secara eksponensial dengan kandungan klorofilnya, baik pada benih segar (basah) maupun kering (Gambar 2). Jalink et al. (1998) juga mendapatkan bahwa mutu benih kubis meningkat saat fluoresen dari klorofil dalam benihnya menurun.

**Gambar 2.** Kandungan klorofil benih kering dan basah berkorelasi secara eksponensial dengan fluoresen klorofilnya selama periode pembentukan benih (21-75 hari setelah berbunga; Suhartanto, 2002).
Beberapa Faktor yang Mempengaruhi Kandungan Klorofil Benih


Mekanisme cahaya mempengaruhi kandungan klorofil masih belum jelas, karena diketahui bahwa cahaya dapat menghambat atau mempercepat proses degradasi klorofil (Biswal dan Biswal, 1984). Pada daun padi, degradasi klorofil dihambat oleh penyinaran yang kontinyu dengan intensitas rendah (0.5 μmol photon.m⁻².detik⁻¹), namun dengan intensitas lebih dari 10 μmol photon.m⁻².detik⁻¹ proses penghambatan tersebut berkurang atau laju degradasi klorofil meningkat (Okada et al., 1992). Suhartanto (2002) melaporkan bahwa proses degradasi klorofil pada benih tomot masih terjadi meskipun benih sudah dikeringkan. Benih yang disimpan dalam ruang simpan dengan cahaya merah menurun kandungan klorofilnya, namun bila disimpan di ruang gelap kandungan klorofilnya relatif tetap. Hal yang menarik ialah daya simpan benih dalam ruangan dengan cahaya merah lebih baik dibanding di ruang gelap. Diduga klorofil dari benih dapat menjadi sumber radikal bebas yang dapat mempercepat penurunan viabilitas benih.


Asam abisat (ABA) dan gibberelin (GAs) sangat berperan dalam perkembangan benih (Bewley dan Black, 1994). ABA dan GA endogen mempengaruhi kandungan klorofil benih. Benih tomat yang defisien GA memiliki kandungan klorofil yang lebih tinggi dibanding tetuanya (wild type), sedangkan benih yang defisien ABA memiliki kandungan klorofil paling rendah (Suhartanto, 2002). Hal ini kemungkinan dapat disebabkan oleh adanya hubungan proses biosintesis ABA, GAs dan klorofil. Defisiensi GAs setelah terjadinya hambatan dalam tahap spesifik dalam biosintesis GA akan mengakibatkan perubahan pigmentasi, baik klorofil maupun karotenoid. Lebih lanjut Maluf et al. (1997) menunjukkan bahwa pada mutan benih jagung yang defisien ABA juga akan mengalami defisensi klorofil dan karotenoid. Mereka juga menunjukkan bahwa mutan ini memiliki ekspresi gen rol-rolan pirofosfat sintase yang rendah. Enzim ini bertanggung jawab dalam proses sintesis geranil-geranil pirofosfat, yang merupakan precursor dari ABA, karotenoid dan klorofil. Lebih lanjut dilaporkan bahwa benih mutan yang memiliki kandungan ditokrom rendah ( PHYC defisient mutant ) akan memiliki kandungan klorofil yang rendah pula dan benih dari mutan ini memiliki dormansi yang tinggi.

Peran Klorofil dalam Benih

Sedikit sekali informasi tentang peran dan fungsi klorofil dalam benih. Sugimoto et al. (1987) menunjukkan bahwa benih kedelai yang sedang tumbuh memiliki aktivitas fotosintesis. Hilangnya kemampuan untuk berfotosintesis diduga disebabkan oleh klorofil, yang merupakan precursor dari ABA, karotenoid dan klorofil. Benih yang defisien ABA akan memiliki kloroplas benih akibat terjadinya akumulasi zat-zat cadangan makanan selama periode pemasakan benih.


Berdasarkan berbagai informasi di atas dapat disimpulkan bahwa: (1) klorofil dibutuhkan dalam pembentukan benih, namun sangat tidak diharapkan dalam tahap pemasakan; (2) kehadiran klorofil dalam tahap pemasakan tampaknya berhubungan erat dengan rendahnya mutu benih, khususnya daya simpannya; (3) fluoresensi klorofil benih dapat digunakan sebagai indikator masak fisiologis benih.

DAFTAR PUSTAKA


Nitrogen Transfer of Two Cultivar Faba Bean (*Vicia faba* L.) to Oat (*Avena sativa* L.)

H. Purnamawati
d K. Schmidtke

**ABSTRACT**

Nitrogen fixed by the legume could be used by other plants, such as through rhizodeposition and direct transfer between roots. The possibility of N transfer in intercropping legum-cereal have long been observed, especially legum with short and dense root. This experiment had been carried out to determine whether there was N transfer from faba bean (*Vicia faba* L.) growing in association with oat (*Avena sativa* L.) and whether there was difference between two cultivars of faba bean in the amount of N transfer. Methods used were complete-mixed-root (CMR) between faba bean and oat, and mixed half the root of faba bean with oat (SR). As a tracer isotope $^{15}$N was used in form of K$^{15}$NO$_3$. The experiment was carried out from May 2000 through January 2001 in Institute of Agronomy and Plant Breeding, University of Goettingen Germany. The data of CMR method had great variance. Using SR method a positive value of $^{15}$N enrichment was found in oat indicated that transfer N from faba bean cv. Minica and Scirocco occurred. There was no difference found in the amount of N transferred between the two tested cultivars.

**Key words:** N transfer, $^{15}$N, Faba bean, Oat

**INTRODUCTION**

Because of the ability of legumes to fix N$_2$ from atmosphere through their symbiosis with *Rhizobium* bacteria, legume crops are often included in intercropping systems. Giller and Wilson (1991) described mechanisms by which legume nitrogen can be made available to other plants, included rhizodeposition, root and nodule senescence, and direct transfer of N between roots. Nitrogen transfer from legume to cereal might have the potential for further manipulation to increase cereal yields (Vandermeer, 1989).

Addition yield from mixtures of non legumes and faba beans compared to mean yield of sole crops are often to be found (Jensen, 1986; Bulson et al., 1997; Li et al., 1999). Li et al. (1999) suggested that the beneficial effect of faba bean/maize mixtures was the results of transfer of substances from faba bean to maize via roots. In the field studies neither Danse et al. (1987), who used the $^{15}$N dilution method, nor Cochran and Schlentner (1995), who using N difference method, found an evidence of N transfer from faba bean to cereals. The roots of faba bean are relatively large and sparse so that sloughing of legume roots is not provided. On the other hand, N transfer to non-legume could be proved for numerous grain legumes such as pea (Jensen, 1996) and soybean (Van Kessel et al., 1985; Martin et al., 1991; Hamel et al., 1991).

The proportion of N, which transferred from legume to non-legume plants, depends on the ability of N$_2$ fixation by the legume (Ta and Faris, 1987) and the growing condition of the legume (Giller and Wilson, 1991). Since species or cultivar of legume plants, which have different growth habit, may have a different capacity to fix N, it is likely that the proportion of N transfer could be also different between species and cultivars.

Faba bean is a wildly cultivated grain legume, it occupies nearly 2.3 x 10$^6$ha world-wide (FAO, 2000). There is a lack of information on N transfer and the difference of N transfer from different cultivars of faba bean. Therefore, the following experiment had been carried out with the aim: to determine whether there was N transfer from faba bean, to measure the proportion of N transferred from faba bean to companion plant oat (*Avena sativa* L) and to examine whether there are differences between cultivars of faba bean in the amount of N transfer.

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MATERIAL AND METHODS

Plants, Nutrients and Growth Conditions

The experiment has been carried out at the beginning of summer, in the glasshouse of the Institute of Agronomy and Plant Breeding, University of Goettingen, from May 2000 through January 2001. At that time temperature in the glasshouse was at 15.9 - 29.0 °C and relative humidity of 34.8 - 62.7 %. Two cultivars of faba bean (cv. Minica and Scirocco) were used as donor plants and on. Uplants and on. Uoat (cv. was used as receiver plant. Faba bean cv. Minica had big seed and cv. Scirocco had smaller seed. A nutrient solution (Vincent, 1970) containing basic nutrient, but without N, was given every two days together with pouring. In experiment 1 plants were planted in vermiculite, an N-free media, and in experiment 2 a clay loam soil from the university experimental field was used only in receiver pot. The soil contained for about 0.15 % total N and 1.64 % total C with pH 6.30.

Experimental Designs

The contribution of faba bean-N to the N nutrition of companion plant was studied using two technique named as the complete-mixture root (CMR) and split root (3R). All treatments in these experiments have been arranged in randomised complete block design with six replications.

Complete-Mixture Root

Plants were grown in PVC-pot holding 12 litre N-free vermiculite. One day after the root emergence in moist filter paper, seedlings of faba bean and oat were transplanted to the experiment units. Before planting, seedlings of faba bean were inoculated with Rhizobium. Twelve seedlings of faba bean cv. Minica were planted in a circle and one seedling of oat was planted in the centre (treatment M). The same treatment was done to faba bean cv Scirocco (treatment S). To see whether there was N transported through air, oat was planted alone in 12-litre-PVC pot and twelve plants of legume were placed nearby in six 5-litre-PVC pots (treatment m and s). Control of unlabelled fertilized (C1) and labelled fertilized-oat growing alone (C2) were also included.

Three weeks after transplanting, all pots, except treatment C1 and pots of faba bean in treatment s, were fertilized with 10 mg N, 5 atom % 15N as K15NO3. Pots C1 was fertilized with the same amount of N using unlabelled KNO3.

Split Root

The split root technique used is based on Jensen (1996), who worked with pea and barley. Faba bean was grown with the root divided between two pots. The experiment unit was a 2-litre pot which contained only moist-N-free vermiculite (‘Donor’ pot) and a 5-litre pot as ‘Receiver’ pot which contained a mixture of soil and vermiculite (1:1 %)

Seeds were germinated in N-free vermiculite, which have been washed with deionized water before being transplanted into the experimental units. Four days after sowing, when the radicle root had 5 cm long, the major part of the radicle was cut to promote the lateral roots to grow. The upper 2 cm of the root coming into root was left intact. Then, seedlings were replanted and grown in N-free-vermiculite for further 12 days to allow the plants to produce the lateral roots before being transplanted into the experimental units. Half part of faba bean root was placed in ‘Donor’ pot and the other part in ‘Receiver’ pot. One seedling of oat (one week old) was planted in ‘Receiver’ pot, 6 cm apart from faba bean. As control, one seedling of oat was planted alone in a 5-litre pot. Ten days later, half of pots ‘Donor’ received 20 mg N, 5 atom % 15N (as KNO3). The amount of 5 atom % 15N was added every 3 days to the ‘Donor’ pot for continuous labelling. The other half of pots ‘Donor’ and oat growing alone as control plants were fertilised with non 15N-enriched KNO3 with the same amount of N.

Harvest and Analytical Methods

Plants harvested 90 days after transplanting. In experiment 1 estimation of N transfer will be calculated based on the shoot-N because the difficulty to separate roots of faba bean growing together with oat. In experiment 2, plants were separated into shoot and root. This was possible because the roots of faba bean and oat have different colour. Faba bean roots have darker colour then the oat roots. The shoots and roots of the plants were washed with deionized water, dried at 60°C for 24 hours, weighted, grounded to ≤ 2 mm and analyzed for total N and 15N/14N ratio (Finnigan MAT 251) at Isotope Laboratory for Biological and Medical Research of the University of Goettingen. Before calculating N transfer, the 15N excess of the plants was calculated with the 15N enrichment of oat growing alone as control plant.

Calculation of N Transfer

Estimation of faba bean N contribution to total N of oat in experiment 1 was calculated through comparing the 15N of labelled N fertilizer and 15N units uptake found in oat in treatment M, m, S and s. If the value of 15N units uptake of oat in treatment M, m, S and s were found to be lower than 15N of labelled N uptake, then faba bean N contribution to oat was calculated.
fertilizer, it indicates that there was some amount of nitrogen transferred from legume to oat. The value of $\delta^{15}N$ units uptake was calculated using equation:

$$\delta^{15}N \text{ units uptake} = \frac{\delta^{15}N \text{ unit receiver} - \delta^{15}N \text{ unit control (CI)}}{\text{total N receiver} - \text{total N (CI)}}$$

whereas $\delta^{15}N$ unit (receiver or control) was:

$$\delta^{15}N \text{ unit} = \delta^{15}N \times \text{total N}$$

In the experiment 2, the transfer of N was estimated using a method known as ‘Donor root enrichment’ (Giller et al., 1991). The proportion of N in the receiver plant derived from donor plant root ($%N_{\text{root}}$) was calculated under assumption that N from donor plant deposited in the rhizosphere and taken up by the receiver plant had the same $\delta^{15}N$ enrichment during the labelling period as the donor root at the time of harvest.

$$%N_{\text{root}} = \frac{\text{atom } %^{15}N_{\text{excess receiver plant}}}{\text{atom } %^{15}N_{\text{excess donor root}}} \times 100$$

The amount of N transferred (mg plant $^{-1}$) is calculated as:

$$N_{\text{transferred}} = %N_{\text{root}} \times \text{total N receiver}$$

$$%N_{\text{transfer}} = \frac{N_{\text{transferred}}}{(\text{total N donor} + N_{\text{transferred}})} \times 100$$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry Matter (mg)</th>
<th>Total N uptake (mg)</th>
<th>$\delta^{15}N$ ($^0r_{eo}$)</th>
<th>N content (%)</th>
<th>$\delta^{15}N$ uptake ($^0r_{eo}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (control $^{15}N$)</td>
<td>170</td>
<td>1.01</td>
<td>68,208</td>
<td>0.59</td>
<td>4348.230</td>
</tr>
<tr>
<td>C2 (control $^{15}N$)</td>
<td>220</td>
<td>1.51</td>
<td>4366.095</td>
<td>0.65</td>
<td>15817.665</td>
</tr>
<tr>
<td>OM</td>
<td>170</td>
<td>2.68</td>
<td>2278.638</td>
<td>1.12</td>
<td>19630.479</td>
</tr>
<tr>
<td>OS</td>
<td>210</td>
<td>1.42</td>
<td>3939.530</td>
<td>0.65</td>
<td>15817.665</td>
</tr>
<tr>
<td>Om</td>
<td>370</td>
<td>1.91</td>
<td>5585.948</td>
<td>0.72</td>
<td>19630.479</td>
</tr>
<tr>
<td>Os</td>
<td>200</td>
<td>1.37</td>
<td>4348.230</td>
<td>0.71</td>
<td>16133.405</td>
</tr>
</tbody>
</table>

Note: OM = oat growing together with faba bean cv. Minica
OS = oat growing together with faba bean cv. Scirocco
Om = oat growing surrounded by faba bean cv. Minica
Os = oat growing surrounded by faba bean cv. Scirocco

In experiment 2 faba bean was 16 days older than oat. Although the oat grew poorly, oat in the ‘Receiver’ pot did not get any additional N fertilizer. The source of N for oat, solely, came from soil and N, which deposited by faba bean. The half root of faba bean growing in ‘Donor’ pot was not included for calculating dry matter weight, total N uptake and $\delta^{15}N$ enrichment. Since this half root was directly contacted to N fertilizer, it would add a considerable amount of N and $\delta^{15}N$ to the whole plant basis.

RESULTS AND DISCUSSION

The data of experiment 1 was found to have greatly variance within replications, therefore only the mean values were showed in Table 1.

The oat growing together with faba beans showed the lowest $\delta^{15}N$ value, but it presumably as a result of competition, since total N uptake was lower than that from oat growing alone surrounded by faba (treatment m and s). If the intercropped non legume had less $^{15}N$ as well as more total N than the sole crop non legume it would indicate that the $^{15}N$ had been diluted from the legume (Martin et al., 1991). The $\delta^{15}N$ uptake of oat growing together with cv. Minica was much lower than $\delta^{15}N$ value of labelled N fertilizer. The lower value of $\delta^{15}N$ uptake could be caused by uptake N from mineralization (Giller and Wilson, 1991). During the first weeks of this experiment, faba beans were transplanted twice because the microclimate inside the glasshouse was not favorable. This situation could lead to mineralization of decayed-seed.
Table 2. Plants dry matter, total N uptake and weighted % $^{15}$N excess of oat growing together with faba bean fertilized with or without $^{15}$N.

<table>
<thead>
<tr>
<th></th>
<th>Dry matter (mg plant$^{-1}$)</th>
<th>Total N uptake (mg plant$^{-1}$)</th>
<th>Weighted % $^{15}$N excess</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>plant</td>
</tr>
<tr>
<td>Oat (+ Minica $^{15}$N)</td>
<td>1875</td>
<td>695</td>
<td>2552</td>
</tr>
<tr>
<td>Oat (+ Minica $^{15}$N)</td>
<td>1870</td>
<td>1028</td>
<td>2898</td>
</tr>
<tr>
<td>HSD (0.05)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Oat (+ Scirocco $^{15}$N)</td>
<td>2152</td>
<td>810</td>
<td>2962</td>
</tr>
<tr>
<td>Oat (+ Scirocco $^{15}$N)</td>
<td>2177</td>
<td>1215</td>
<td>3392</td>
</tr>
<tr>
<td>HSD (0.05)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: ns = no significant
Oat (+ Minica $^{15}$N) = Oat growing together with cv. Minica fertilized by KNO$_3$
Oat (+ Minica $^{15}$N) = Oat growing together with cv. Minica fertilized by K$_2$NO$_3$
Oat (+ Scirocco $^{15}$N) = Oat growing together with cv. Scirocco fertilized by KNO$_3$
Oat (+ Scirocco $^{15}$N) = Oat growing together with cv. Scirocco fertilized by K$_2$NO$_3$

% $^{15}$N excess was based on the difference between $^{15}$N enrichment of oat growing together with faba beans and oat control.

Growing together with faba bean did not influence oat growth, but reduced total N uptake of oat and shoot-N was much more affected than root-N (Table 3). Between the two cultivars of faba bean, Minica had larger shoot dry weight and tended to accumulate more shoot-N ($P = 0.090$) than Scirocco. There was a tendency that oat growing together with Minica had a lower N uptake compared to the oat growing with Scirocco.

Since oat did not directly fertilized with $^{15}$N, the positive value of $^{15}$N enrichment found in shoot and root of oat was an indication that N had been transferred from faba beans to oat. Oat growing together with Minica had significantly more $^{15}$N enrichment than oat growing together with Scirocco (Table 3). The higher $^{15}$N excess in oat growing with Minica appeared more likely, because of oat growing together with Minica took up less N compared to the oat growing with Scirocco, rather than because of Minica transferred more N than Scirocco. Table 4 showed that the proportion of N in oat that came from Minica was not significantly higher than the proportion of N came from Scirocco.

Table 3. Plants dry matter, N uptake and weighted $^{15}$N excess of oat and faba bean.

<table>
<thead>
<tr>
<th></th>
<th>Dry matter (mg plant$^{-1}$)</th>
<th>Total N uptake (mg plant$^{-1}$)</th>
<th>Weighted % $^{15}$N excess</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>plant</td>
</tr>
<tr>
<td>oat control</td>
<td>2425</td>
<td>1080</td>
<td>3505</td>
</tr>
<tr>
<td>oat (+ Minica)</td>
<td>1870</td>
<td>1028</td>
<td>2898</td>
</tr>
<tr>
<td>oat (+ Scirocco)</td>
<td>2177</td>
<td>1215</td>
<td>3392</td>
</tr>
<tr>
<td>HSD (0.05)</td>
<td>6815  a</td>
<td>3790</td>
<td>10,605</td>
</tr>
<tr>
<td>Scirocco (+ oat)</td>
<td>5562  b</td>
<td>3747</td>
<td>9,308</td>
</tr>
<tr>
<td>HSD (0.05)</td>
<td>0.036</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: ns = no significant.
Numbers with different letters indicates significant difference.
% $^{15}$N excess was based on the difference between $^{15}$N enrichment of oat growing together with faba bean and oat control.
Table 4. Estimated N-transfer\(^1\) from faba bean cv Minica and Scirocco to oat.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Donor root enrichment method</th>
<th>N transferred (mg N plant(^1))</th>
<th>N(\text{fix root})(^3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat growing</td>
<td></td>
<td>1.280</td>
<td>2.51</td>
</tr>
<tr>
<td>With Minica</td>
<td></td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Oat growing</td>
<td></td>
<td>0.927</td>
<td>1.62</td>
</tr>
<tr>
<td>With Scirocco</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HSD(0.05) ns ns ns

Note:  
1) = calculation for plant excluded the half root in pot ‘donor’  
2) = N transferred as percentage of faba bean-N  
3) = N transferred as percentage of oat-N  
ns = no significant.

Despite the low N nutrition condition created in ‘Receiver’ pot to enhance faba bean-N-fixation, there seemed no immediate benefit of N to oat. At the time of harvest, Minica contained in average 1.98% N and Scirocco 1.99% N. This N uptake of faba beans was roughly three times of total N in oat but only less than 0.68% of faba bean's N was transferred to oat. Giller et al. (1991) and Jensen (1996) found that the amounts of N transferred from bean to companion plant in intercropping might improve under a severe limitation on the growth of the beans such as an insect attack or shading.

We found no correlation between the amount of N, which transferred to oat, neither to the amount of N taken up by faba beans, nor the amount of N left in the media of 'Receiver' pot after harvest.

Jensen (1996) explained that the donor root enrichment method may give the most reliable estimation of N transfer in continuous split root labelling because the \(^{15}\text{N}\) enrichment of donor root is probably similar than the \(^{15}\text{N}\) enrichment of N deposited. But, we must also consider that at 90 days after sowing, faba bean was at the end of pod filling stage. The \(^{15}\text{N}\) enrichment of faba beans roots might be different with the \(^{15}\text{N}\) enrichment of N deposited and taken up by oat since decomposition and senescence of nodules and root had been occurred.

We concluded that transfer of N from faba bean to associated plant does occur, although the amount of N transferred was very small (less than 0.68% of faba bean's N). The result of experiment also showed that there was no difference in the amount of N transfer between the two tested cultivars, Minica and Scirocco.

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REFERENCES


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