Chapter 4

Introgression of WUE and root traits in mulberry and identification of superior genotypes
Chapter 4
Introgression of water use efficiency and root traits in mulberry and identification of improved lines

4.1 Introduction

Plants have naturally evolved several adaptive mechanisms for survival under water-limited conditions. From the agronomic point of view, the concept of drought tolerance is linked to better growth rate under water deficit conditions. In this context, for any trait to be useful, it should benefit one of the functional components in the biological model for yield (Passioura 1986). To respond to these challenges, one of the options is to introduce new genes so that many interesting traits which otherwise not present together in crop populations can be incorporated. Indeed, a large amount of genetic diversity and many interesting traits for breeding (strong tolerance to abiotic and biotic stresses) are not present in cultivated forms of crop species and may be found in the germplasm including wild relatives of the crop species. It has been demonstrated in many cases that interspecific crosses reveal transgressive variation among the progeny. The transgressive segregants with favorable phenotypes can be used immediately as progenitors in a breeding program and they can also be further characterized to understand the genetic basis of transgressive variation. We propose here to develop genetic and genomic resources that would allow breeders and geneticists to take advantage of this unexploited reservoir of genetic diversity.

Mulberry is a heterozygous crop, where the allelic variation is significantly high. Therefore, identification of alleles controlling specific traits is extremely difficult. The genetic control of the traits can only be located in the genome by raising the trait-specific mapping population by crossing the contrasting parental types for the desired traits followed by mapping and QTL analysis. The task of bringing together two unrelated traits in a single genetic background is termed as introgression and the individuals are called introgressed lines (ILs). As discussed in earlier two chapters, water use efficiency and root traits are the most important components of drought tolerance mechanism in plants. High WUE and high root type can be hybridized to obtain superior recombinants for improved performance towards moisture stress tolerance. Once an association is established between moisture stress tolerance and a specific ILs, it is possible to use advanced forward and reverse approaches for genetic analysis to mine down and identify the gene(s) that are directly involved (Yano 2001; Jander et al. 2002), but it is also feasible to use the ILs directly in a breeding program without knowing the identity of the gene(s) that are involved (Gur and Zamir 2004).
Introgressing the traits of interest can be followed using molecular markers that are mapped flanking or tightly linked with the traits of interest. The use of MAS facilitates a faster introgression since plants can be sampled and genotypes with target traits can be identified even at the early stage of development (Jonaliza et al. 2007). In introgression breeding programs, a general similarity to the recipient line is sought. This similarity is too general to be gauged by the level of resemblance in one or several defined quantitative traits. Indeed, such breeding programs have as an aim, the genome in its entirety. Therefore, Hillel et al. (1990) propose the term ‘genomic selection (GS)’ for these processes.

The main objective of the present study was to develop ILs for WUE and rooting in mulberry and to identify superior recombinants with improved performance in moisture stress by accurate phenotyping. The ILs may be used for validation of identified QTLs for WUE and root traits in mulberry.

Objectives:

- Development of introgressed lines for WUE and root traits for validation of identified QTLs for the trait.
Complex phenotypes can be dissected genetically by evaluating and comparing Chromosome Segment Substitution Lines (CSSLs). By linking the information about gene identity with the database, it will be able to provide a dictionary of genes with known function that are contained in each of the CSSLs. This dictionary is also a key ingredient in enabling comparative approaches to the study of phenotype-genotype relationships. Once an association is established between a phenotype and specific introgressed lines (ILs), it is possible to use advanced forward and reverse approaches to genetic analysis to mine down and identify the gene(s) that are directly involved (Yano 2001; Jander et al. 2002), but it is also feasible to use the ILs directly in a breeding program without knowing the identity of the gene(s) that are involved (Gur and Zamir 2004).

Selection against genetic drag can save ten’s of generations (Young and Tanksley 1989), not necessarily at high cost (Hospital 2001). This is true even if the target gene is in fact a QTL located with a given error on the genetic map (Visscher et al. 1996; Hospital and Charcosset 1997). However, the number of targets is then limited. It is generally not possible to introgress more than four or five QTL, even with the largest population sizes. However, this assumes that the QTL is a ‘true’ QTL, that is, it has an effect on the trait of interest (not a false positive) and that this effect is sustained over the breeding programme; specifically, the effect will be unmodified by changes of genetic background, environment or epistatic relationships with other genes. This assumption is not always true.

Marker assisted selection of BC₁F₁ and then BC₂F₁ lines carrying the desirable donor segment with the best possible return to the background of the cultivated variety provide a set of lines offering an optimal distribution of the wild introgressions. Genome-wide introgression of a small fraction of the wild genome species while keeping the genetic background of the cultivated species is a good mean to explore the largely untapped reservoir of useful alleles of interest that remain in the wild species. This is especially interesting for species with narrow genetic base. This approach has been widely utilized for the introgression of favourable QTL(s) for various traits in tomato (Eshed and Zamir 1995; Tanksley et al. 1996; Fulton et al. 1997), rice (Wang et al. 2005; Xu et al. 2005; Zhang et al. 2006), wheat (Liu et al. 2006) and in barley (Korff et al. 2007; Schmalenbach et al. 2009). In peanut, the reproductive barriers between wild and cultivated species, and technical difficulties encountered in making large number of crosses as well as the hot period between sowing and flowering have impeded the application of QTL.

The rate of success starts to decrease for introgression of larger numbers of target QTL. If Stuber (1994) claims success in increasing grain yield in maize lines by introgression of six
favourable chromosome segments, it must be noted that none of the improved lines had all six segments together. Starting with the ILs of Eshed and Zamir (1995), Lawson et al. (1997) introgressed four target chromosomal regions containing five QTL for pest resistance (acyl-sugar accumulation) from wild tomato into cultivated tomato. The introgression of the four regions was successful at the genomic level. However, the level of acyl-sugar accumulation in the progeny introgressed for the five QTL was lower than expected, and, in particular, lower than that of the inter-specific F1 hybrid.

Toojinda et al. (1998) successfully introgressed two QTL for stripe rust resistance in barley into a genetic background different from the one used to map the QTL. The effects of both QTL were confirmed and additional QTL were detected in the new background, including some resistance alleles brought in by the susceptible parent. It should be considered as more than successful from the breeder’s perspective. However, would someone concerned with a more fundamental understanding of the genetic bases of quantitative traits consider such unexpected results ‘successful’? Probably the answer relies on which genes were polymorphic in both populations. Chee et al. (2001) also reported the successful transfer of a QTL for grain protein concentration in wheat into a different genetic background. Ahmadi et al. (2001) successfully introgressed two QTL for resistance to yellow mottle virus in rice. Yousef and Juvik (2002) selected three markers linked to QTL that enhanced seedling emergence in sweet corn.

Backcrossing isolates, a gene or chromosomal region in a different genetic background (the genetic background of the recurrent parent), helps to dissect the genetic architecture of quantitative traits. In fact, it is one of the few reliable methods to validate the additive effect of a QTL or candidate gene after it is putatively detected. In addition, backcrossing could be used prior to detection to increase the precision of QTL mapping or exploit wild genetic resources. Introgressing one gene into a different genetic background removes or modifies the possible epistatic interactions between that gene and the rest of the genome; it is then useful to study the additive (non-epistatic) effect of the gene. Combinations of ILs with different genes and different backgrounds can thus be used to study epistatic interactions. In introgression breeding programs, a general similarity to the recipient line is sought. This similarity is too general to be gauged by the level of resemblance in one or several defined quantitative traits. Indeed, such breeding programs have as an aim the genome in its entirety.

Marker assisted introgression is not always successful. One major limitation is not the ability of marker-based selection to produce the selection objective at the molecular level - all experiments report that genomic composition of the produced genotype is close to that predicted by theory. Rather, success of introgression depends on the ability of the target genes to exhibit the
expected effects once introgressed in a new genetic background (the genetic background of the recurrent parent). Introgression alters the epistatic interactions between the target and the donor background. Then, it is essentially the additive effect of the target that can show up in the new background, unless new epistatic interactions are built up. Hence, the ‘success’ or ‘failure’ of introgression experiments may help discover whether QTL effects are mostly additive. Conversely, such experiments could be ideal for determining the extent to which epistasis affects the genetic architecture of complex traits. However, when the target QTL fails to exhibit the expected additive effect, it can be that its effect was epistatic (i.e. non-additive) or that the QTL had no effect at all (i.e. it was a false positive). The first obvious reason is that the putative QTL may in fact be a false positive. As wisely stated by Bernardo (2004) among others, ‘the false discovery rate (FDR) should be kept low so that resources are not wasted in introgressing false QTL. Perhaps the success or failure in attempts to introgress QTL may be partly due to the s (significance) level used to identify QTL’. In addition, it is known that estimated QTL effects are generally biased for several reasons (Beavis 1994; Bost et al. 2001). Some groups have engaged in an extensive and valuable effort to empirically estimate the repeatability of QTL detection and correct biases in estimated effects (Melchinger et al. 1998; Schon et al. 2004). It might be interesting to perform a quantitative survey of the published results but it does not seem that putative QTL displaying has no effect after introgression is the most frequent case. Many times, the QTL is still detected after introgression but its effect is reduced. In the worst cases, it is even opposite to the expected effect, as in Bouchez et al. (2002). Such cases may not be explained completely by statistical error or imprecision. Another potential cause of unexpected results is the possibility of QTL by environment interactions, which have definitely been shown to exist in some cases (Ribaut et al. 2002a,b). Generally, genotype x environment interactions are frequent in plants but less so in animals. Another explanation worthy of greater consideration is the possibility that the chromosomal segments detected as QTL hold not just one but several genes. Recombination between those genes would then simply modify the effect of the introgressed segments. Such an observation is actually not infrequent after fine mapping of QTL segments (Eshed and Zamir 1995; Monna et al. 2002; Steinmetz et al. 2002; Christians and Keightley 2004). Finally, the last, though probably not the least, cause of unexpected introgression results is epistasis, either between QTL or between QTL and the genetic background. Epistasis can be beneficial and MAS can be very rewarding in some cases. In an experiment, Ahmadi et al. (2001) confirmed the epistatic relationship between two QTL that were detected in a population. ILs hosting one of the QTL, but not the other, displayed no effect. Conversely, the line hosting both QTL exhibited the expected effect. In such cases, use of marker-
based selection is clearly valuable because manipulating epistatic relationships by phenotypic selection is generally very difficult.

The advanced backcross quantitative trait locus (AB-QTL) analysis (Tanksley and Nelson 1996) is a powerful strategy to exploit and use the potential of wild alleles in breeding programs. This methodology integrates the QTL analysis and the introgression of alleles from wild germplasm into elite material under the assumption that marker regions positively associated with traits of agronomic interest can be identified and transferred into elite cultivars (Bernacchi et al. 1997; Frary et al. 2004). Marker loci associated with favorable wild alleles can be used to select genotypes containing these specific genomic regions. After a few selfing generations, ILs are obtained and can be field-tested and used for variety development (Frary et al. 2004). Since they contain small wild fragments evenly distributed throughout the elite recurrent genome, ILs can be used for genetic and functional genomic studies, such as the dissection of gene functions and map-based cloning of QTLs underlying quantitative and qualitative traits (Li et al. 2004; Tian et al. 2006). In addition, ILs is an important reservoir of alleles that can be used in breeding programs for the development of new cultivars with higher genetic diversity and that are more resistant to biotic and abiotic stresses.

Introgressing the traits of interest can be followed using molecular markers that are mapped flanking or tightly linked with the traits being incorporated. The use of MAS facilitates a faster introgression since plants can be sampled and genotypes with target traits can be identified even at the early stage of development (Jonaliza et al. 2007). There are numerous reports on mapping QTL for yield and other traits, and on using marker assisted selection to introgress QTL. But there are few published reports that critically evaluate using markers to manipulate yield. Stuber et al. (1992) identified QTL alleles that were predicted to increase hybrid yield if introgressed into selected maize (Zea mays L.) inbred lines. Markers were used to introgress the alleles into the inbred lines, and indeed the hybrids from the enhanced inbred lines yielded better than hybrids from inbred lines that lacked the marker-introgressed QTL (Stuber 1994).

To overcome the problem of interspecific mapping of QTL associated with yield, we modified population structures used in the past by combining them with DNA markers (Eshed and Zamir 1995). Wehrhahn and Allard (1965) have demonstrated that effects of individual QTL in wheat can be measured by using backcross inbred lines (BILs). BILs are characterized by the low proportion of the donor parent in each of the population members and therefore are ideally suited for mapping inter-specific variation. Shen et al. (2001) manipulated four QTL for drought resistance (root depth) in rice, a trait that is very difficult to manage phenotypically. Starting from doubled haploid lines, they produced a number of BC$_3$F$_3$ lines, each introgressed for one or two QTL at
most. Among the four QTL, one exhibited the expected effect in the progeny, one was finally revealed as a false positive, one segment was shown to contain two QTL in repulsion phase (C/K) that reduced its expression and one segment did not exhibit the expected effect.

Lecomte et al. (2004) introgressed five QTL strongly involved in tomato fruit quality into three different recipient lines through MAS. The breeding efficiency varied strongly with the recipient parent and significant interactions between QTL and genetic backgrounds were shown for all the studied traits. About 50% of the QTL were confirmed in each new background and new QTL were detected. The QTL with the largest effects were the most stable. Thabuis et al. (2004) transferred resistance to Phytophthora capsici alleles at four QTL from a small fruited pepper into a bell pepper recipient by three cycles of marker-assisted backcrossing. Introgression was successful but a decrease of the effect from the moderate-effect QTL and of the epistatic interaction between QTL was observed. Finally, in some cases, none of the introgressed QTL had any effect, for example, three QTL for grain yield in barley (Kandemir et al. 2000) and three QTL for high yield in soybean (Reyna and Sneller 2001).
4.3 Materials and methods

4.3.1 Plant Material
Hybridization program involving Dudhia White (female, high root type) and MS-3 (male, high WUE type) was undertaken in the month of July 2007 and about 1200 progeny were raised in glass house (Figures - 4.4.1 a & b). Later in June 2008, the fully-grown seedlings were transferred to an experimental plot under paired-row system of plantation – (5+3) x 2, and 1104 introgressed lines (ILs) were established with recommended package of practices (Figures - 4.4.1 c, d & e). The first pruning was done in November 2008 and first data recording was carried out in January 2009.

4.3.2 Visual grading and shoot biomass
Based on the visual observation of different parameters, (viz., branching nature, leaf color, leaf nature, leaf lobation, leaf size, leaf appearance, inter-nodal distances and number of branches) the lines were graded. Besides, shoot biomass was also taken into consideration for the preliminary screening of the ILs.

4.3.3 Gas exchange parameters
Gas exchange parameters viz. photosynthetic rate (A), conductance (gs), intercellular CO₂ concentration (Ci), transpiration rate (Tr), leaf vapor pressure deficit (vpd), leaf temperature (Tleaf), ratio of assimilation rate and stomatal conductance (A/gs) and ratio of intercellular carbon concentration and stomatal conductance (Ci/gs) were recorded for the selected lines using the LI-6400 portable photosynthesis system (LI-COR, USA). The instrument works with the principle of infrared absorption by CO₂ molecules with the extent of IR absorption being proportional to the CO₂ concentration. Similarly, a separate IR analyzer was used for measuring the water vapor concentrations. A leaf chamber automatically controlled to maintain the requisite temperature (by Peltier cooling system) and light intensities using blue and red LEDs were used to clamp a leaf whose gas exchange rates are to be determined. The system measures the difference in the CO₂ and water vapor concentration in the air that enters the leaf chamber.

4.3.4 Carbon isotope discrimination
$\Delta^{13}C$ value was also recorded in the selected ILs using the National Facility for Isotope Ratio Mass Spectrophotometer (IRMS) at University of Agricultural Sciences, Bangalore. The dried leaf powder was weighed (~1 mg) in silver capsules and dropped into an oxidation reactor of the Flash Elemental analyzer (NA1112, Carlo Erba, Italy) using an auto sampler. The element analyzer is
interfaced with the Delta plus Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) through a Continuous flow device (Conflo III). The samples were combusted in the presence of ultra pure oxygen and chromium oxide catalyst. The resultant gases (predominantly CO$_2$, water and nitrous oxide) were then swept into the reduction reactor along with ultra high pure Helium carrier gas. The excess oxygen unused during oxidation would be absorbed and the nitrous oxide would be reduced to molecular nitrogen. The gases are passed through a column of magnesium per chlorate to remove moisture and then through a 1.5 m GC column filled with porapack molecular sieve to separate the CO$_2$ from N$_2$ gas. These gases are then introduced into the ion source of the IRMS to determine the carbon isotopic composition calibrated against PDB standards. A lab standard also calibrated against the same international standard was introduced at regular intervals throughout the analysis to check the sensitivity of the measurement as well as the analytical uncertainty. Deviation of the carbon isotopic composition in the leaf samples from that of the atmospheric air ($\Delta^{13}$C) was computed assuming the $\delta^{13}$C$_{\text{air}}$ to be -8‰ as follows:

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}}{1 + \delta^{13}\text{C}_{\text{plant}}/1000}$$

### 4.3.5 Statistical analysis

The data of different parameters were analyzed statistically and the minimum, maximum, mean, and coefficient of variation were calculated using MS Excel 2003. The frequency distributions of the traits were plotted graphically using plot graph module in MS Excel 2003.
4.4 Results

The seedlings of Dudhia White x MS-3 numbering 1104 were established in a plot measuring 0.4 acres. The plants were allowed to grow for six months and then pruned. Preliminary assessment of the phenotype was made sixty days after 1st pruning to short-list reasonable number of superior introgressed lines (ILs) for further in-depth analysis using gas exchange and $^{13}$C.

Preliminary short-listing (Visual Grading and shoot biomass)

Key morphological characters were visually scored and graded among all the ILs. The morphological branching nature (BN), leaf color (LC) and total shoot biomass (TSB) showed near normal frequency distribution among the progeny. Maximum number of progeny belonged to grade “low” for number of branches. Similarly, maximum numbers of progeny were unlobed; however, a few shallow, medium and deeply lobed progeny were also recorded. Medium sized leaves followed by small ones represented majority of the progeny. Inter-nodal distance (IND) was short in maximum number of progeny. 25% of the total progeny showed glossy and thick leaves. Frequency distributions of important characters are plotted graphically in Figure – 4.4.2.1-2. However, a few of the progeny showed large sized leaves.

The shoot biomass among 1104 lines showed a minimum of 20 g to maximum of 3,180 g with an average of 1,125 g and the coefficient of variation was 34.7%. These observations are critically assessed and based on the cumulative grading and shoot biomass, a total of 63 ILs were short-listed for intensive evaluation by gas exchange and $^{13}$C parameters.

Selection of top ten performing ILs

The statistical summary of the eight gas exchange parameters {viz. photosynthetic rate (A), conductance (gs), intercellular CO2 concentration (Ci), transpiration rate (Tr), leaf vapor pressure deficit (vpd), leaf temperature (Tleaf), ratio of assimilation rate and stomatal conductance (A/gs) and Ratio of intercellular carbon concentration and stomatal conductance (Ci/gs)}, $^{13}$C and TSB is provided in the Table – 4.4.1 for the top 63 short-listed ILs.

Gas exchange parameters such as gs, Ci/gs and A/gs showed high coefficient of variation (CV). Total shoot biomass ranged from 620 to 2040 g with a mean of 1370 g. Minimum CV was recorded in Tleaf (2.94) and $^{13}$C (2.86). All these parameters were critically evaluated and the top ten performing ILs were finally selected for further use as pre-breeding resources (Table – 4.4.2). Alternatively, top five performing ILs viz. IL - 126, 280, 309, 311 and 314 have also been identified for further evaluation under preliminary evaluation trial for moisture stress in mulberry.
4.5 Discussion

Water use efficiency (WUE) is one of the important aspects of moisture stress tolerance in plants. However, one cannot always expect to increase the productivity of the plant by increasing the WUE alone (Sheshshayee et al. 2003). As detailed in Chapter 1, significant variability related to WUE already exists in mulberry and hence breeding for the trait can be rewarding. According to the yield model of Passioura (1976), the total transpiration from the plant canopy is another important factor contributing to the enhanced productivity in plants. The factors that contribute to total transpiration are the rate and transpired area and, it is the reflection of root traits. In other words, significant improvement in the crop productivity can be achieved only when WUE of the plant is enhanced without substantial reduction in total transpiration in any condition of water availability (stress or non-stress). It is also understood that a high transpiration is normally maintained in plants that have the ability to harness water from deeper soil profiles. To summarize, if deep-rooted mulberry plants also possess moderately high WUE, significant gain in growth and productivity can be achieved in mulberry.

Though high WUE can be achieved by decreasing transpiration rate (T), such an approach is not desirable. In such plant species, often referred to as ‘conductance types’, increase in the WUE has an advantage when moisture is not a limitation. But in other crops, termed as ‘capacity types’ (variability brought about by difference in photosynthetic efficiency), breeding for WUE results in higher biomass under both water limited and unlimited conditions. In these types, WUE will be higher without any reduction in transpiration (Udayakumar et al. 1998b). In nature, it is extremely rare to find such a situation. Introgresion of WUE and factors contributing to higher transpiration (high root types) are thought to be some of the solutions to bring these traits or QTLs contributing to such traits in a single genetic background. In this direction, efforts were made to identify ‘high types’ for both WUE and rooting in mulberry (Chapter 1). In the present study, 1104 introgressed lines (ILs) were developed by hybridizing Dudhia White (high root type) and MS-3 (high WUE type). These lines were evaluated by visual scoring followed by grading along with total shoot biomass (TSB) to short-list a practically feasible number for further in-depth analysis by gas exchange parameters, $\Delta C$ and TSB. Variability among 63 short-listed ILs is shown in the Table – 4.4.1. Variability of short-listed ILs is expected because these lines were originated from heterozygous parental background. The top ten ILs were selected mainly based on $\Delta C$ (range
16.97 – 18.44), TSB (range 1.14 – 2.04 kg) and other qualitative parameters of the leaf. However, the gas exchange parameters, which are highly influenced by the soil moisture, temperature, sunlight etc., were only used as a supportive data to arrive at the final list. Further, five top lines were identified for possible preliminary evaluation under moisture stress condition.

The study had three major goals. First is to bring the two divergent attributes of plant system namely, the WUE and higher transpiration rate to common genetic background so that a mulberry plant type can be productive under moisture limited condition would be evolved. Secondly, to develop pre-breeding resources in mulberry drought tolerance so that mulberry breeders can take such improvement program confidently. As the breeders can be certain about the genes contributing to such traits can transfer them into a popular variety using conventional crop improvement methods. The productivity of the top five ILs can be assessed under both preliminary and final evaluation trials in moisture stress environments for direct utilization in mulberry cultivation. Lastly, these lines can be utilized for validating the QTLs (specifically the major ones) for associated traits in mulberry. The work is expected to pave way for further research on QTLs associated with moisture stress tolerance and their manifestation in mulberry.
Figure 4.4.2.1: Frequency distribution of the six morphological characters among the 1104 introgression (WUE and root) lines
Figure 4.4.2.2: Frequency distribution of the four morphological and important quantitative traits among the 1104 introgression (WUE and root) lines.
Figure 4.4.1 a, b, c, d & e: Where (a) and (b) are introgressed lines raised in the glass house and (c), (d) and (e) depicts the establishment of the same in the experimental plot.
Table 4.4.2: Top ten selected introgressed lines along with the listing of important parameters

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Progeny No.</th>
<th>Branching nature</th>
<th>Leaf color</th>
<th>Leaf nature</th>
<th>Leaf lobation</th>
<th>Leaf size</th>
<th>Leaf appearance</th>
<th>Leaf thickness</th>
<th>Inter-nodal distance</th>
<th>No. of branches</th>
<th>Total biomass (Kg)</th>
<th>D13C (‰)</th>
<th>Photo (µmol m⁻² s⁻¹)</th>
<th>Cond (mol m⁻² s⁻¹)</th>
<th>Ci (mmol)</th>
<th>Tr (mmol m⁻² s⁻¹)</th>
<th>VpdL (kPa)</th>
<th>Tleaf (°C)</th>
<th>A/ gs</th>
<th>Ci/ gs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL-8</td>
<td>E</td>
<td>DG</td>
<td>HM</td>
<td>UL</td>
<td>L</td>
<td>NGL</td>
<td>TK</td>
<td>S</td>
<td>LO</td>
<td>1.14</td>
<td>17.57</td>
<td>16.50</td>
<td>0.33</td>
<td>271.33</td>
<td>5.39</td>
<td>1.53</td>
<td>28.01</td>
<td>50.46</td>
<td>829.77</td>
</tr>
<tr>
<td>2</td>
<td>IL-126</td>
<td>SE</td>
<td>G</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>NGL</td>
<td>TK</td>
<td>S</td>
<td>M</td>
<td>1.76</td>
<td>17.96</td>
<td>21.37</td>
<td>0.70</td>
<td>300.67</td>
<td>8.33</td>
<td>1.15</td>
<td>27.59</td>
<td>30.44</td>
<td>428.30</td>
</tr>
<tr>
<td>3</td>
<td>IL-228</td>
<td>E</td>
<td>DG</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>GL</td>
<td>TK</td>
<td>S</td>
<td>LO</td>
<td>1.72</td>
<td>18.06</td>
<td>16.73</td>
<td>0.53</td>
<td>300.33</td>
<td>7.81</td>
<td>1.40</td>
<td>28.62</td>
<td>31.77</td>
<td>570.25</td>
</tr>
<tr>
<td>4</td>
<td>IL-237</td>
<td>SE</td>
<td>DG</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>NGL</td>
<td>TK</td>
<td>S</td>
<td>LO</td>
<td>1.52</td>
<td>18.05</td>
<td>22.10</td>
<td>0.46</td>
<td>270.00</td>
<td>7.82</td>
<td>1.59</td>
<td>29.97</td>
<td>47.87</td>
<td>584.84</td>
</tr>
<tr>
<td>5</td>
<td>IL-265</td>
<td>SE</td>
<td>G</td>
<td>HM</td>
<td>UL</td>
<td>L</td>
<td>NGL</td>
<td>TN</td>
<td>S</td>
<td>M</td>
<td>1.78</td>
<td>18.44</td>
<td>18.43</td>
<td>0.45</td>
<td>285.33</td>
<td>6.97</td>
<td>1.46</td>
<td>28.91</td>
<td>41.05</td>
<td>635.49</td>
</tr>
<tr>
<td>6</td>
<td>IL-280</td>
<td>E</td>
<td>G</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>NGL</td>
<td>TN</td>
<td>S</td>
<td>LO</td>
<td>1.86</td>
<td>17.49</td>
<td>21.30</td>
<td>0.42</td>
<td>266.67</td>
<td>7.62</td>
<td>1.71</td>
<td>30.51</td>
<td>51.28</td>
<td>642.05</td>
</tr>
<tr>
<td>7</td>
<td>IL-307</td>
<td>E</td>
<td>G</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>GL</td>
<td>TK</td>
<td>S</td>
<td>LO</td>
<td>1.32</td>
<td>16.97</td>
<td>17.00</td>
<td>0.41</td>
<td>285.67</td>
<td>6.65</td>
<td>1.50</td>
<td>29.23</td>
<td>42.34</td>
<td>691.13</td>
</tr>
<tr>
<td>8</td>
<td>IL-309</td>
<td>E</td>
<td>G</td>
<td>HM</td>
<td>UL</td>
<td>L</td>
<td>GL</td>
<td>TK</td>
<td>S</td>
<td>LO</td>
<td>2.04</td>
<td>18.01</td>
<td>18.33</td>
<td>0.51</td>
<td>292.33</td>
<td>7.58</td>
<td>1.42</td>
<td>28.98</td>
<td>36.28</td>
<td>578.50</td>
</tr>
<tr>
<td>9</td>
<td>IL-311</td>
<td>E</td>
<td>G</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>GL</td>
<td>TN</td>
<td>S</td>
<td>M</td>
<td>1.88</td>
<td>17.88</td>
<td>15.87</td>
<td>0.33</td>
<td>275.00</td>
<td>5.98</td>
<td>1.66</td>
<td>29.67</td>
<td>47.50</td>
<td>823.35</td>
</tr>
<tr>
<td>10</td>
<td>IL-314</td>
<td>SE</td>
<td>DG</td>
<td>HM</td>
<td>UL</td>
<td>M</td>
<td>NGL</td>
<td>TK</td>
<td>S</td>
<td>LO</td>
<td>1.84</td>
<td>17.54</td>
<td>17.70</td>
<td>0.48</td>
<td>291.33</td>
<td>7.09</td>
<td>1.40</td>
<td>28.61</td>
<td>36.98</td>
<td>608.64</td>
</tr>
</tbody>
</table>

**Table 4.4.1:** Statistical summary of total biomass and other important traits of selected introgressed lines (63 Nos.)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Traits</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shoot biomass (Kg)</td>
<td>0.62</td>
<td>2.04</td>
<td>1.37</td>
<td>0.32</td>
<td>23.50</td>
</tr>
<tr>
<td>2</td>
<td>$\Delta^{13}\text{C}$ (%o)</td>
<td>16.97</td>
<td>20.23</td>
<td>18.58</td>
<td>0.72</td>
<td>3.86</td>
</tr>
<tr>
<td>3</td>
<td>A (mol m$^{-2}$ s$^{-1}$)</td>
<td>13.33</td>
<td>30.37</td>
<td>21.75</td>
<td>3.62</td>
<td>16.64</td>
</tr>
<tr>
<td>4</td>
<td>gs (mol m$^{-2}$ s$^{-1}$)</td>
<td>0.21</td>
<td>1.11</td>
<td>0.58</td>
<td>0.19</td>
<td>32.61</td>
</tr>
<tr>
<td>5</td>
<td>Ci (mmol)</td>
<td>249.00</td>
<td>324.33</td>
<td>284.70</td>
<td>17.20</td>
<td>6.04</td>
</tr>
<tr>
<td>6</td>
<td>Tr (mmol m$^{-2}$ s$^{-1}$)</td>
<td>4.41</td>
<td>10.40</td>
<td>7.83</td>
<td>1.19</td>
<td>15.26</td>
</tr>
<tr>
<td>7</td>
<td>vpd (kPa)</td>
<td>0.83</td>
<td>1.96</td>
<td>1.35</td>
<td>0.23</td>
<td>17.32</td>
</tr>
<tr>
<td>8</td>
<td>Tleaf ($^\circ$C)</td>
<td>27.58</td>
<td>31.82</td>
<td>29.96</td>
<td>0.88</td>
<td>2.94</td>
</tr>
<tr>
<td>9</td>
<td>A/gs</td>
<td>17.96</td>
<td>64.72</td>
<td>40.05</td>
<td>9.93</td>
<td>24.79</td>
</tr>
<tr>
<td>10</td>
<td>Ci/gs</td>
<td>291.32</td>
<td>1208.74</td>
<td>540.65</td>
<td>175.60</td>
<td>32.48</td>
</tr>
</tbody>
</table>