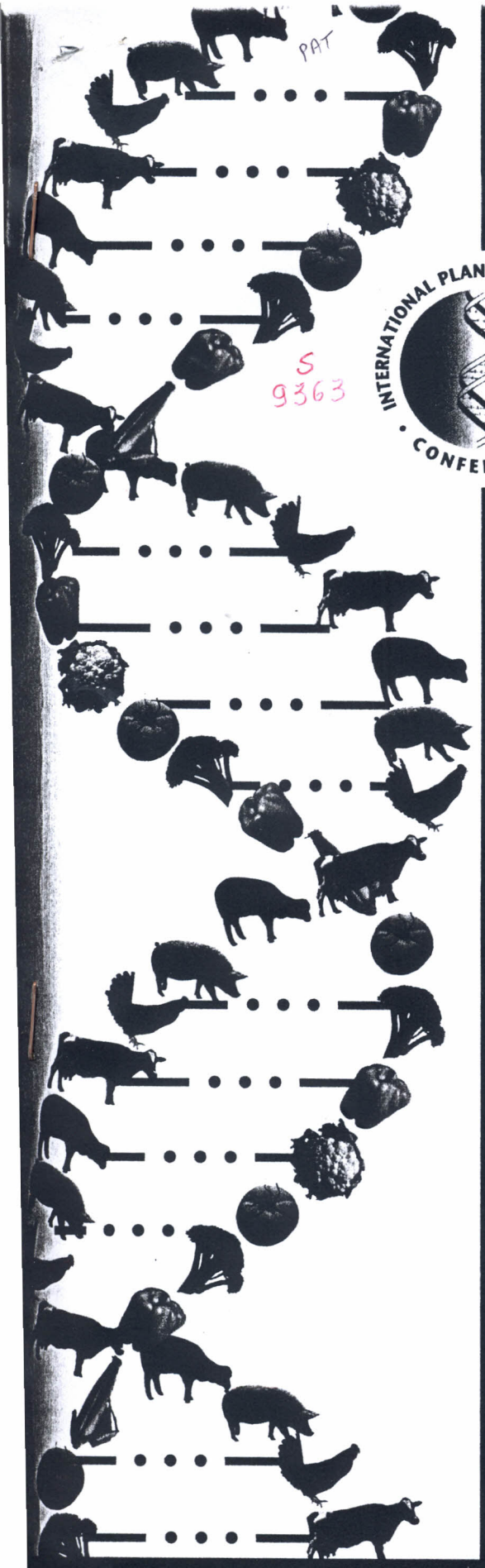

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FINAL ABSTRACTS GUIDE

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AFLP ANALYSIS OF GENETIC DIVERSITY WITHIN AND BETWEEN POPULATIONS OF PERENNIAL RYEGRASS (LOLIUM PERENNE L.)

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The agriculturally important forage grass perennial ryegrass exhibits an obligate outbreeding habit, ensuring high levels of genetic heterogeneity. Cultivars are currently distinguished on the basis of morphological characteristics that are highly influenced by environmental and developmental effects, posing a problem for consistent cultivar identification. DNA-based molecular markers are unaffected by environmental factors and may be used to obtain information on the distinctiveness, uniformity and stability of cultivars, to provide a means for seed purity certification and the protection of plant breeders rights. Amplified Fragment Length Polymorphisms (AFLPs) were used to study genetic diversity within and between perennial ryegrass populations. Genetic variation has been determined within and between four populations with different breeding histories and within and between three populations derived from low numbers of parents (restricted genetic base). Bulking individuals of a population was also evaluated and profiles were consistent with observations based on individual analysis. AFLP-profiling can be used to discriminate between closely related, restricted base cultivars even when varieties are closely related. AFLP-profiling will provide a useful tool for cultivar identification and the support of plant breeders rights.

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ANALYSIS OF THE GENETIC DIVERSITY WITHIN THE CARICA GENUS USING AFLP

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During previous ethnobotanical and agronomical studies in Ecuador, an unknown and undescribed variability within the plant genus Carica was observed. The genus is best known by the tropical species Carica papaya, but in South America and particularly in Ecuador other species from more temperate zones (up to 2800 m) are also used as fruit trees, in the forest or semi-domesticated in gardens. The last taxonomic revision recognizes 21 species (11 in Ecuador), but is far from complete and shows several gaps. In a recent study two species are newly recorded for Ecuador, and a completely new species is being described. In 1997 two other species were discovered as novel for Ecuador. To obtain more detailed knowledge about the phylogenetic relationships of the Carica genus, data on the degree of the genetic diversity within the population are necessary. Numerous molecular marker techniques have proven their high value in population biology, taxonomy, ‡ Several techniques have been described, but based on the two most important characteristics of molecular markers (multiplex ratio and information content), AFLP is the most suitable technique for our objectives. The data derived from the AFLP-fingerprints, used as the input for specific software programs (Treeconw, NTSYS-pc), leads to easily interpretable graphics. The aim of applying this technique is to describe genotypes of Carica races, as well as their genotypic diversity (percentage of difference, dynamics and distribution of genotypes). Finally this analysis will allow the testing of supposed races as hypotheses based on morphological observations, directly on their genotype.

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SEQUENCING OF SPECIFIC AFLP AMPLICONS REVEALS VERY HIGHLY CONSERVED SEQUENCES IN TWO UNRELATED F2 POPULATIONS OF CARROT

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Amplified fragment length polymorphism (AFLP) is a fast and reliable tool to generate a large number of DNA markers. In two unrelated F2 population of carrot (Daucus carota), Brasilia x HCM and B493 x QAL (wild carrot), we hypothesized that 1) DNA digested with the same restriction endonuclease enzymes and amplified with the same primer combination and 2) sharing the same position in acrylamide gel should show conserved sequences of DNA. Re-amplification of AFLP fragments from acrylamide gels was done using standard elution protocols. Analysis of these PCR products in agarose gels revealed unique and isolated paired bands. AFLP fragments were purified from agarose gels with standard purification kits, cloned into plasmids using Topo cloning kit and sequenced with fluorescent ddNTPs. Among 10 paired fragments sequenced from each F2 population, 6 have shown high similarity and the same number of nucleotides, 1 with same size but different sequences, and 3 with different sizes (less than 12 nucleotides) and sequences. Of all SCAR (sequence characterized amplified regions) primers tested, 15% have resulted in co-dominant markers in both populations. We hypothesize that sequences of AFLP amplicons should be useful for designing specific probes not only to generate co-dominant markers but also to study different species in a broader evolutionary perspective.

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cDNA-AFLP ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN THE FLOWER BUDS OF PHALAENOPSIS HSING FEI cv. H. F. AND ITS SOMACLONAL VARIANT

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The flower of Phalaenopsis Hsing Fei cv. H. F. has bronze color and elegant style, and its somaclonal variant has mosaic color and its lip development is often distorted. Using cDNA- amplified fragment length polymorphism (cDNA-AFLP) technology, we compared the fingerprints of mRNA samples extracted from the mature flower buds of P. Hsing Fei cv. H. F. and its somaclonal variant. cDNA samples were digested with Taq I, which recognizes a 4-bp nucleotide, and amplified with a combination of two sets of primers. One set of primers are with a single selective nucleotide at its 3'-end and the other with three selective nucleotides at its 3'-end and a radio-labeled 5'-end. Approximately 3200 fragments were amplified after PCR with 32 primer combinations. Among these, 27 fragments were differentially expressed between P. Hsing Fei cv. H. F. and its somaclonal variants. Fifteen amplified fragments were specific for the P. Hsing Fei cv. H. F., and the other 12 fragments were specific for the variant. Sequence analysis showed that three of P. Hsing Fei cv. H. F.-specific transcripts have 51%~92%homology with RNA-dependent RNA polymerase of Cymbidium mosaic virus. One variant-specific transcript showed 70% identity in amino acid level to the mutator-like transposase of Arabidopsis. These differential gene expression may lead to the mosaic flower color and distorted lip morphogenesis of the variant.