

Chittaranjan Kole *Editor*

Wild Crop Relatives: Genomic and Breeding Resources Millets and Grasses

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 Springer

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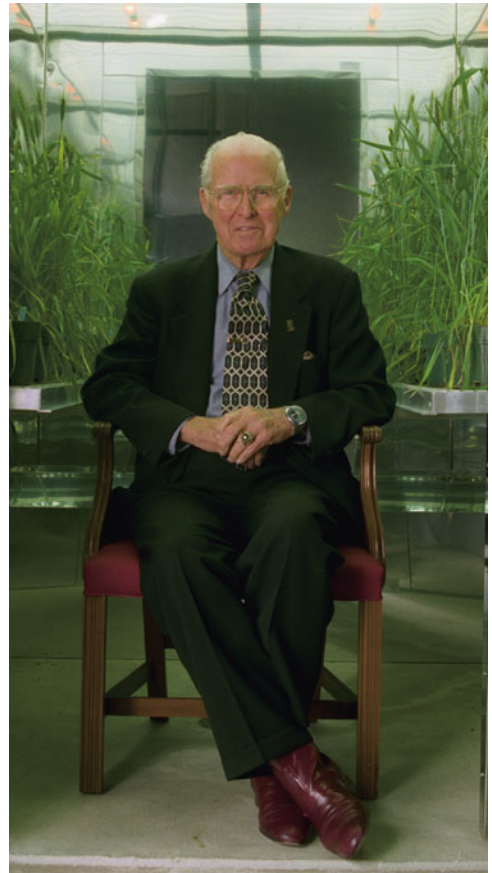
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Dedication

Dr. Norman Ernest Borlaug,¹ the Father of Green Revolution, is well respected for his contributions to science and society. There was or is not and never will be a single person on this Earth whose single-handed service to science could save millions of people from death due to starvation over a period of over four decades like Dr. Borlaug's. Even the Nobel Peace Prize he received in 1970 does not do such a great and noble person as Dr. Borlaug justice. His life and contributions are well known and will remain in the pages of history of science. I wish here only to share some facets of this elegant and ideal personality I had been blessed to observe during my personal interactions with him.

It was early 2007 while I was at the Clemson University as a visiting scientist one of my lab colleagues told me that “somebody wants to talk to you; he appears to be an old man”. I took the telephone receiver casually and said hello. The response from the other side was – “I am Norman Borlaug; am I talking to Chitta?” Even a million words would be insufficient to define and depict the exact feelings and thrills I experienced at that moment!



¹The photo of Dr. Borlaug was kindly provided by Julie Borlaug (Norman Borlaug Institute for International Agriculture, Texas A&M Agriculture) the granddaughter of Dr. Borlaug.

I had seen Dr. Borlaug only once, way back in 1983, when he came to New Delhi, India to deliver the Coromandal Lecture organized by Prof. M.S. Swaminathan on the occasion of the 15th International Genetic Congress. However, my real interaction with him began in 2004 when I had been formulating a 7-volume book series entitled *Genome Mapping and Molecular Breeding in Plants*. Initially, I was neither confident of my ability as a series/book editor nor of the quality of the contents of the book volumes. I sent an email to Dr. Borlaug attaching the table of contents and the tentative outline of the chapters along with manuscripts of only a few sample chapters, including one authored by me and others, to learn about his views as a source of inspiration (or caution!) I was almost sure that a person of his stature would have no time and purpose to get back to a small science worker like me. To my utter (and pleasant) surprise I received an email from him that read: “May all Ph.D.’s, future scientists, and students that are devoted to agriculture get an inspiration as it refers to your work or future work from the pages of this important book. My wholehearted wishes for a success on your important job”. I got a shot in my arm (and in mind for sure)! Rest is a pleasant experience – the seven volumes were published by Springer in 2006 and 2007, and were welcome and liked by students, scientists and their societies, libraries, and industries. As a token of my humble regards and gratitude, I sent Dr. Borlaug the Volume I on *Cereals and Millets* that was published in 2006. And here started my discovery of the simplest person on Earth who solved the most complex and critical problem of people on it – hunger and death.

Just one month after receiving the volume, Dr. Borlaug called me one day and said, “Chitta, you know I cannot read a lot now-a-days, but I have gone through only on the chapters on wheat, maize and rice. Please excuse me. Other chapters of this and other volumes of the series will be equally excellent, I believe”. He was highly excited to know that many other Nobel Laureates including Profs. Arthur Kornberg, Werner Arber, Phillip Sharp, Günter Blobel, and Lee Hartwell also expressed generous comments regarding the utility and impact of the book series on science and the academic society. While we were discussing many other textbooks and review book series that I was editing at that time, again in my night hours for the benefit of students, scientists, and industries, he became emotional and said to me, “Chitta, forget about your original contributions to basic and applied sciences, you deserved Nobel Prize for Peace like me for providing academic foods to millions of starving students and scientists over the world particularly in the developing countries. I will recommend your name for the World Food Prize, but it will not do enough justice to the sacrifice you are doing for science and society in your sleepless nights over so many years. Take some rest Chitta and give time to Phullara, Sourav and Devleena” (he was so particular to ask about my wife and our kids during most of our conversations). I felt honored but really very ashamed as I am aware of my almost insignificant contribution in comparison to his monumental contribution and thousands of scientists over the world are doing at least hundred-times better jobs than me as scientist or author/editor of books! So, I was unable to utter any words for a couple of minutes but realized later that he must been too affectionate to me and his huge affection is the best award for a small science worker as me!

In another occasion he wanted some documents from me. I told him that I will send them as attachments in emails. Immediately he shouted and told me: “You know, Julie (his granddaughter) is not at home now and I cannot check email myself. Julie does this for me. I can type myself in type writer but I am not good in computer. You know what, I have a xerox machine and it receives fax also. Send me

the documents by fax”. Here was the ever-present child in him. Julie emailed me later to send the documents as attachment to her as the ‘xerox machine’ of Dr. Borlaug ran out of ink!

Another occasion is when I was talking with him in a low voice, and he immediately chided me: “You know that I cannot hear well now-a-days; I don’t know where Julie has kept the hearing apparatus, can’t you speak louder?” Here was the fatherly figure who was eager to hear each of my words!

I still shed tears when I remember during one of our telephone conversations he asked: “You know I have never seen you, can you come to Dallas in the near future by chance?” I remember we were going through a financial paucity at that time and I could not make a visit to Dallas (Texas) to see him, though it would have been a great honor.

In late 2007, whenever I tried to talk to Dr. Borlaug, he used to beckon Julie to bring the telephone to him, and in course of time Julie used to keep alive all the communications between us when he slowly succumbed to his health problems.

The remaining volumes of the *Genome Mapping and Molecular Breeding in Plants* series were published in 2007, and I sent him all the seven volumes. I wished to learn about his views. During this period he could not speak and write well. Julie prepared a letter based on his words to her that read: “Dear Chitta, I have reviewed the seven volumes of the series on *Genome Mapping and Molecular Breeding in Plants*, which you have authored. You have brought together genetic linkage maps based on molecular markers for the most important crop species that will be a valuable guide and tool to further molecular crop improvements. Congratulations for a job well done”.

During one of our conversations in mid-2007, he asked me what other book projects I was planning for Ph.D. students and scientists (who had always been his all-time beloved folks). I told him that the wealth of wild species already utilized and to be utilized for genetic analysis and improvement of domesticated crop species have not been deliberated in any book project. He was very excited and told me to take up the book project as soon as possible. But during that period I had a huge commitment to editing a number of book volumes and could not start the series he was so interested about.

His sudden demise in September 2009 kept me so morose for a number of months that I could not even communicate my personal loss to Julie. But in the meantime, I formulated a 10-volume series on *Wild Crop Relatives: Genomic and Breeding Resources* for Springer. And whom else to dedicate this series to other than Dr. Borlaug!

I wrote to Julie for her formal permission and she immediately wrote me: “Chitta, Thank you for contacting me and yes I think my grandfather would be honored with the dedication of the series. I remember him talking of you and this undertaking quite often. Congratulations on all that you have accomplished!” This helped me a lot as I could at least feel consoled that I could do a job he wanted me to do and I will always remain grateful to Julie for this help and also for taking care of Dr. Borlaug, not only as his granddaughter but also as the representative of millions of poor people from around the world and hundreds of plant and agricultural scientists who try to follow his philosophy and worship him as a father figure.

It is another sad experience of growing older in life that we walk alone and miss the affectionate shadows, inspirations, encouragements, and blessings from the fatherly figures in our professional and personal lives. How I wish I could treat my next generations in the same way as personalities like Mother Teresa and Dr. Norman Borlaug and many other great people from around the world treated me!

During most of our conversations he used to emphasize on the immediate impact of research on the society and its people. A couple of times he even told me that my works on molecular genetics and biotechnology, particularly of 1980s and 1990s, have high fundamental importance, but I should also do some works that will benefit people immediately. This advice elicited a change in my thoughts and workplans and since then I have been devotedly endeavoring to develop crop varieties enriched with phytomedicines and nutraceuticals. Borlaug influenced both my personal and professional life, particularly my approach to science, and I dedicate this series to him in remembrance of his great contribution to science and society and for all his personal affection, love and blessings for me.

I emailed the above draft of the dedication page to Julie for her views and I wish to complete my humble dedication with great satisfaction with the words of Julie who served as the living ladder for me to reach and stay closer to such as great human being as Dr. Borlaug and express my deep regards and gratitude to her. Julie's email read: "Chitta, Thank you for sending me the draft dedication page. I really enjoyed reading it and I think you captured my grandfather's spirit wonderfully. . . . So thank you very much for your beautiful words. I know he would be and is honored."

Clemson, USA

Chittaranjan Kole

Preface

Wild crop relatives have been playing enormously important roles both in the depiction of plant genomes and the genetic improvement of their cultivated counterparts. They have contributed immensely to resolving several fundamental questions, particularly those related to the origin, evolution, phylogenetic relationship, cytological status and inheritance of genes of an array of crop plants; provided several desirable donor genes for the genetic improvement of their domesticated counterparts; and facilitated the innovation of many novel concepts and technologies while working on them directly or while using their resources. More recently, they have even been used for the verification of their potential threats of gene flow from genetically modified plants and invasive habits. Above all, some of them are contributing enormously as model plant species to the elucidation and amelioration of the genomes of crop plant species.

As a matter of fact, as a student, a teacher, and a humble science worker I was, still am and surely will remain fascinated by the wild allies of crop plants for their invaluable wealth for genetics, genomics and breeding in crop plants and as such share a deep concern for their conservation and comprehensive characterization for future utilization. It is by now a well established fact that wild crop relatives deserve serious attention for domestication, especially for the utilization of their phytomedicines and nutraceuticals, bioenergy production, soil reclamation, and the phytoremediation of ecology and environment. While these vastly positive impacts of wild crop relatives on the development and deployment of new varieties for various purposes in the major crop plants of the world agriculture, along with a few negative potential concerns, are envisaged the need for reference books with comprehensive deliberations on the wild relatives of all the major field and plantation crops and fruit and forest trees is indeed imperative. This was the driving force behind the inception and publication of this series.

Unlike the previous six book projects I have edited alone or with co-editors, this time it was very difficult to formulate uniform outlines for the chapters of this book series for several obvious reasons. Firstly, the status of the crop relatives is highly diverse. Some of them are completely wild, some are sporadically cultivated and some are at the initial stage of domestication for specific breeding objectives recently deemed essential. Secondly, the status of their conservation varies widely: some have been conserved, characterized and utilized; some have been eroded completely except for their presence in their center(s) of origin; some are at-risk or endangered due to genetic erosion, and some of them have yet to be explored. The third constraint is the variation in their relative worth, e.g. as academic model, breeding resource, and/or potential as “new crops.”

The most perplexing problem for me was to assign the chapters each on a particular genus to different volumes dedicated to crop relatives of diverse crops grouped based on their utility. This can be exemplified with *Arabidopsis*, which has primarily benefited the Brassicaceae crops but also facilitated genetic analyses and improvement in crop plants in other distant families; or with many wild relatives of forage crops that paved the way for the genetic analyses and breeding of some major cereal and millet crops. The same is true for wild crop relatives such as *Medicago truncatula*, which has paved the way for in-depth research on two crop groups of diverse use: oilseed and pulse crops belonging to the Fabaceae family. The list is too long to enumerate. I had no other choice but to compromise and assign the genera of crop relatives in a volume on the crop group to which they are taxonomically the closest and to which they have relatively greater contributions. For example, I placed the chapter on genus *Arabidopsis* in the volume on oilseeds, which deals with the wild relatives of Brassicaceae crops amongst others.

However, we have tried to include deliberations pertinent to the individual genera of the wild crop relatives to which the chapters are devoted. Descriptions of the geographical locations of origin and genetic diversity, geographical distribution, karyotype and genome size, morphology, etc. have been included for most of them. Their current utility status – whether recognized as model species, weeds, invasive species or potentially cultivable taxa – is also delineated. The academic, agricultural, medicinal, ecological, environmental and industrial potential of both the cultivated and/or wild allied taxa are discussed.

The conservation of wild crop relatives is a much discussed yet equally neglected issue albeit the in situ and ex situ conservations of some luckier species were initiated earlier or are being initiated now. We have included discussions on what has happened and what is happening with regard to the conservation of the crop relatives, thanks to the national and international endeavors, in most of the chapters and also included what should happen for the wild relatives of the so-called new, minor, orphan or future crops.

The botanical origin, evolutionary pathway and phylogenetic relationship of crop plants have always attracted the attention of plant scientists. For these studies morphological attributes, cytological features and biochemical parameters were used individually or in combinations at different periods based on the availability of the required tools and techniques. Access to different molecular markers based on nuclear and especially cytoplasmic DNAs that emerged after 1980 refined the strategies required for precise and unequivocal conclusions regarding these aspects. Illustrations of these classical and recent tools have been included in the chapters.

Positioning genes and defining gene functions required in many cases different cytogenetic stocks, including substitution lines, addition lines, haploids, monoloids and aneuploids, particularly in polyploid crops. These aspects have been dealt in the relevant chapters. Employment of colchicoidy, fluorescent or genomic in situ hybridization and Southern hybridization have reinforced the theoretical and applied studies on these stocks. Chapters on relevant genera/species include details on these cytogenetic stocks.

Wild crop relatives, particularly wild allied species and subspecies, have been used since the birth of genetics in the twentieth century in several instances such as studies of inheritance, linkage, function, transmission and evolution of genes. They have been frequently used in genetic studies since the advent of molecular markers. Their involvement in molecular mapping has facilitated the development of mapping

populations with optimum polymorphism to construct saturated maps and also illuminating the organization, reorganization and functional aspects of genes and genomes. Many phenomena such as genomic duplication, genome reorganization, self-incompatibility, segregation distortion, transgressive segregation and defining genes and their phenotypes have in many cases been made possible due to the utilization of wild species or subspecies. Most of the chapters contain detailed elucidations on these aspects.

The richness of crop relatives with biotic and abiotic stress resistance genes was well recognized and documented with the transfer of several alien genes into their cultivated counterparts through wide or distant hybridization with or without employing embryo-rescue and mutagenesis. However, the amazing revelation that the wild relatives are also a source of yield-related genes is a development of the molecular era. Apomictic genes are another asset of many crop relatives that deserve mention. All of these past and the present factors have led to the realization that the so-called inferior species are highly superior in conserving desirable genes and can serve as a goldmine for breeding elite plant varieties. This is particularly true at a point when natural genetic variability has been depleted or exhausted in most of the major crop species, particularly due to growing and promoting only a handful of so-called high-yielding varieties while disregarding the traditional cultivars and landraces. In the era of molecular breeding, we can map desirable genes and polygenes, identify their donors and utilize tightly linked markers for gene introgression, mitigating the constraint of linkage drag, and even pyramid genes from multiple sources, cultivated or wild taxa. The evaluation of primary, secondary and tertiary gene pools and utilization of their novel genes is one of the leading strategies in present-day plant breeding. It is obvious that many wide hybridizations will never be easy and involve near-impossible constraints such as complete or partial sterility. In such cases gene cloning and gene discovery, complemented by intragenic breeding, will hopefully pave the way for success. The utilization of wild relatives through traditional and molecular breeding has been thoroughly enumerated over the chapters throughout this series.

Enormous genomic resources have been developed in the model crop relatives, for example *Arabidopsis thaliana* and *Medicago truncatula*. BAC, cDNA and EST libraries have also been developed in some other crop relatives. Transcriptomes and metabolomes have also been dissected in some of them. However, similar genomic resources are yet to be constructed in many crop relatives. Hence this section has been included only in chapters on the relevant genera.

In this book series, we have included a section on recommendations for future steps to create awareness about the wealth of wild crop relatives in society at large and also for concerns for their alarmingly rapid decrease due to genetic erosion. The authors of the chapters have also emphasized on the imperative requirement of their conservation, envisaging the importance of biodiversity. The importance of intellectual property rights and also farmers' rights as owners of local landraces, botanical varieties, wild species and subspecies has also been dealt in many of the chapters.

I feel satisfied that the authors of the chapters in this series have deliberated on all the crucial aspects relevant to a particular genus in their chapters.

I am also very pleased to present many chapters in this series authored by a large number of globally reputed leading scientists, many of whom have contributed to the development of novel concepts, strategies and tools of genetics, genomics and breeding and/or pioneered the elucidation and improvement of particular plant

genomes using both traditional and molecular tools. Many of them have already retired or will be retiring soon, leaving behind their legacies and philosophies for us to follow and practice. I am saddened that a few of them have passed away during preparation of the manuscripts for this series. At the same time, I feel blessed that all of these stalwarts shared equally with me the wealth of crop relatives and contributed to their recognition and promotion through this endeavor.

I would also like to be candid with regard to my own limitations. Initially I planned for about 150 chapters devoted to the essential genera of wild crop relatives. However, I had to exclude some of them either due to insignificant progress made on them during the preparation of this series, my failure to identify interested authors willing to produce acceptable manuscripts in time or authors' backing out in the last minute, leaving no time to find replacements. I console myself for this lapse with the rationale that it is simply too large a series to achieve complete satisfaction on the contents. Still I was able to arrange about 125 chapters in the ten volumes, contributed by nearly 400 authors from over 40 countries of the world. I extend my heartfelt thanks to all these scientists, who have cooperated with me since the inception of this series not only with their contributions, but also in some cases by suggesting suitable authors for chapters on other genera. As happens with a mega-series, a few authors had delays for personal or professional reasons, and in a few cases, for no reason at all. This caused delays in the publication of some of the volumes and forced the remaining authors to update their manuscripts and wait too long to see their manuscripts in published form. I do shoulder all the responsibilities for this myself and tender my sincere apologies.

Another unique feature of this series is that the authors of chapters dedicated to some genera have dedicated their chapters to scientists who pioneered the exploration, description and utilization of the wild species of those genera. We have duly honored their sincere decision with equal respect for the scientists they rightly reminded us to commemorate.

Editing this series was, to be honest, very taxing and painstaking, as my own expertise is limited to a few cereal, oilseed, pulse, vegetable, and fruit crops, and some medicinal and aromatic plants. I spent innumerable nights studying to attain the minimum eligibility to edit the manuscripts authored by experts with even life-time contributions on the concerned genera or species. However, this indirectly awakened the "student-for-life" within me and enriched my arsenal with so many new concepts, strategies, tools, techniques and even new terminologies! Above all, this helped me to realize that individually we know almost nothing about the plants on this planet! And this realization strikingly reminded me of the affectionate and sincere advice of Dr. Norman Borlaug to keep abreast with what is happening in the crop sciences, which he used to do himself even when he had been advised to strictly limit himself to bed rest. He was always enthusiastic about this series and inspired me to take up this huge task. This is one of the personal and professional reasons I dedicated this book series to him with a hope that the present and future generations of plant scientists will share the similar feelings of love and respect for all plants around us for the sake of meeting our never-ending needs for food, shelter, clothing, medicines, and all other items used for our basic requirements and comfort. I am also grateful to his granddaughter, Julie Borlaug, for kindly extending her permission to dedicate this series to him.

I started editing books with the 7-volume series on Genome Mapping and Molecular Breeding in Plants with Springer way back in 2005, and I have since

edited many other book series with Springer. I always feel proud and satisfied to be a member of the Springer family, particularly because of my warm and enriching working relationship with Dr. Sabine Schwarz and Dr. Jutta Lindenberg, with whom I have been working all along. My special thanks go out to them for publishing this “dream series” in an elegant form and also for appreciating my difficulties and accommodating many of my last-minute changes and updates.

I would be remiss in my duties if I failed to mention the contributions of Phullara – my wife, friend, philosopher and guide – who has always shared with me a love of the collection, conservation, evaluation, and utilization of wild crop relatives and has enormously supported me in the translation of these priorities in my own research endeavors – for her assistance in formulating the contents of this series, for monitoring its progress and above all for taking care of all the domestic and personal responsibilities I am supposed to shoulder. I feel myself alien to the digital world that is the sine qua non today for maintaining constant communication and ensuring the preparation of manuscripts in a desirable format. Our son Sourav and daughter Devleena made my life easier by balancing out my limitations and also by willingly sacrificing the spare amount of time I ought to spend with them. Editing of this series would not be possible without their unwavering support.

I take the responsibility for any lapses in content, format and approach of the series and individual volumes and also for any other errors, either scientific or linguistic, and will look forward to receiving readers’ corrections or suggestions for improvement.

As I mentioned earlier this series consists of ten volumes. These volumes are dedicated to wild relatives of Cereals, Millets and Grasses, Oilseeds, Legume Crops and Forages, Vegetables, Temperate Fruits, Tropical and Subtropical Fruits, Industrial Crops, Plantation and Ornamental Crops, and Forest Trees.

This volume “Wild Crop Relatives – Genomic and Breeding Resources: Millets and Grasses” includes 16 chapters dedicated to *Agrostis*, *Bromus*, *Cenchrus*, *Cynodon*, *Dactylis*, *Dichanthium*, *Eleusine*, *Eragrostis*, *Festuca*, *Lolium*, *Panicum*, *Paspalum*, *Pennisetum*, *Phleum*, *Setaria* and *Zoysia*. The chapters of this volume were authored by 48 scientists from 11 countries of the world namely Algeria, Argentina, Australia, France, India, Japan, New Zealand, Poland, Portugal, Turkey, and the USA.

It is my sincere hope that this volume and the series as a whole will serve the requirements of students, scientists and industries involved in studies, teaching, research and the extension of millets and grasses with an intention of serving science and society.

Clemson, USA

Chittaranjan Kole

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Abbreviations

5S	rDNA gene coding for the 5S rRNA
A/L	Accumulation/loss mechanism
ABA	Abscisic acid
ACCase	Acetyl coenzyme A carboxylase
AFLP	Amplified fragment length polymorphism
ALS	Acetolactate synthase
ANTC	Anthocyanin
APO	Aposporous locus
ASGR	Apospory-specific genomic region
ASGR	Apomixis-related gene region
AusPGRIS	Australian Plant Genetic Resource Information Service
BAC	Bacterial artificial chromosome
BADH	Betaine-aldehyde dehydrogenase
BC	Backcross
BP	Before present
BSA	Bulked segregant analysis
CAD	Cinnamyl alcohol dehydrogenase
CCoAOMT	Caffeoyl-CoA O-methyltransferase
CCR	Cinnamoyl CoA reductase
cDNA	Complementary DNA
CENARGEN	Centro Nacional de Pesquisa em Recursos Genéticos e Biotecnologia
CGIAR	Consultative Group on International Agricultural Research
CHCH	Coiled-coil-helix-coiled-coil-helix
CIAT	International Centre for Tropical Agriculture
CID	Carbon isotope discrimination
CISP	Conserved intron scanning primers
cM	Centi-Morgan
CMS	Cytoplasmic male sterility
COMT	Caffeic acid O-methyltransferase
COMT	Caffeic acid/5-hydroxyferulic acid O-methyltransferase
cpDNA	Chloroplast-DNA
CPPSE	Centro de Pesquisa de Pecuária do Sudeste
CP-sHSP	Chloroplast-localized small HSP
<i>Crs-1</i>	Creeping bentgrass specific-1 gene
CTAB	Cetyl trimethyl ammonium bromide
cTBP	Combinatorial tubulin-based polymorphism

cv.	Cultivar
CWR	Crop wild relative
DAF	DNA amplification fingerprinting
DAPI	4',6-Diamidino-2-phenylindole
DDBJ	DNA Data Bank of Japan
DEF	Diferentially expressed fragment
DM	Dry matter
EMBL	European Molecular Biology Laboratory
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
EST	Expressed sequence tag
FAA	Formalin: acetic acid: ethyl alcohol
FAME	Fatty acid methyl ester
FISH	Fluorescence in situ hybridization
GAI	Gene of agronomic interest
GBSSI	Granule-bound starch synthase I
GC	Gas chromatography
GFP	Green fluorescent protein
GI	Genes index
GIS	Genomic in situ hybridization
GMM	Genotype matrix mapping
GR	Glyphosate resistant
GRIN	Germplasm Resource Information Network (USDA-ARS)
GSS	Genome survey sequence
hph	Hygromycin phosphotransferase (gene)
<i>HSP</i>	Heat shock protein
IBC	Institute of Biodiversity Conservation
IBONE	Instituto de Botánica del Nordeste
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IGER	Institute of Grassland and Environmental Research
IGFRI	Indian Grassland and Fodder Research Institute
ILRI	International Livestock Research Institute
IMI	Imidazolinone
INDEL	Insertion/deletion
INTA	National Institute of Agriculture of Argentina
ISSR	Inter-simple sequence repeat
ITS	Internal transcribed spacer
IVDMD	In vitro dry matter digestibility
LEA	Late embryogenesis abundant
LG	Linkage group
LLR	Leucine-rich repeat
MAS	Marker-assisted selection
MDA	Malondialdehyde
mRNA	Messenger-RNA
NADP	Nicotinamide adenine dinucleotide phosphate
NADP-ME	NADP-malic enzyme
NBPGR	National Bureau of Plant Genetic Resources
NBS	Nucleotide binding site
NCBI	National Center for Biotechnology Information
NDF	Neutral detergent fiber

<i>ndhF</i>	NADH dehydrogenase F
<i>nirK</i> , <i>nirS</i>	Nitrite reductase genes
NMDH	NADP-dependent malate dehydrogenase
NOR	Nucleolar organizer region
<i>nosZ</i>	Nitrous oxide reductase gene
NPGS	National Plant Germplasm System
NPK	Nitrogen phosphorus potassium
OMT	<i>O</i> -Methyl transferase
PAC	Plasmid artificial chromosome
PACC	Panicoid-arundinoid-chloridoid-centothecoid
PAL	Phenylalanine ammonialyase
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PD	Power of discrimination
PGR	Plant growth regulator
PHYB	Phytochrome B
PIC	Polymorphic information content
PSII	Photosystem II
Q-PCR	Quantitative real-time PCR
QTA	Quantitative trait allele
QTL	Quantitative trait loci
QTN	Quantitative trait nucleotide
R gene	Resistance gene
RAPD	Random(ly) amplified polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
RGL	R-gene-like sequence
RIL	Recombinant Inbred Line
RNAi	RNA-interference
RT-PCR	Real-time quantitative PCR
RWC	Relative water content
s.str.	sensu stricto
SC	Secondary constriction
SCAR	Sequence-characterized amplified region
SDRF	Single dose restriction fragment
SINGER	System-wide Information Network for Genetic Resources
SNP	Single nucleotide polymorphism
SRAP	Sequence-related amplified polymorphism
SSR	Simple sequence repeat
STS	Sequence tagged site
TBP	Tubulin-based polymorphism
TDF	Transcript derived fragment
TNL	TIR-NBS-LRR
tRNA	Transfer-RNA
<i>uidA</i>	β -Glucuronidase (gene)
UNED	United Nations Conference on Environment and Development
UPGMA	Unweighted pair group method with arithmetic mean

USDA	United States Department of Agriculture
USDA-APHIS	USDA-Animal and Plant Health Inspection Service
USDA-SCS	USDA Soil Conservation Service
USGA	United States Golf Association
WUE	Water use efficiency

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Chapter 1

Agrostis

B.S. Ozdemir and H. Budak

1.1 Introduction

The grass family (Poaceae) emerged 60 million years ago (Kellogg 2001) and it is one of the largest families that include various species with high economical importance, especially the essential cereal crops that are obligate in the daily diet. Grasses are used for many purposes such as in food production, industry, lawns, and sports fields. Turfgrasses are used in sports fields and recreation areas whereas they prevent soil erosion in natural habitats. In the United States, turfgrass constitutes the second place in the seed market (Lee 1996).

Agrostis spp., bentgrass, contains more than 200 perennial turfgrass species as a genus in the Poaceae family (Hitchcock 1971), but five species of this genus are mainly used as turfgrass, which are all out-crossing, perennial, and cool-season grasses: colonial (*Agrostis capillaris* L.), velvet (*Agrostis canina* L.), creeping (*Agrostis stolonifera* L.), redtop (*Agrostis gigantea* Roth), and dryland (*Agrostis castellana* Boiss. and Reut.). *Agrostis* spp. is taxonomically classified under Gramineae (Poaceae) family, Pooideae subfamily, Aveneae tribe, and *Agrostis* genus (Warnke 2003).

Bentgrasses are cool-season grasses that are widely used on golf courses (tees, fairways, and greens) at

temperate regions due to their dense nature, low mowing heights without damage, and green appearance. They are also used for parks and forage. They are cross-pollinating, self-incompatible, and pollinated by wind. *Agrostis* can exhibit both clonal growth and seed reproduction.

It is hard to classify *Agrostis* genus taxonomically. Due to the similar morphological characters among this genus, others features are needed to be included for identification of new germplasms. Laser flow cytometry for the determination of ploidy level was found to be effective in differentiating between diploid, tetraploid, and hexaploid forms by evaluating six *Agrostis* species; *A. canina* L. subsp. *canina*, *A. canina* L. subsp. *montana* (Hartm.) Hartm., *A. stolonifera* var. *palustris* (Huds.) Farw., *A. capillaris* L., *A. castellana* Boiss. & Reut., and *Agrostis alba* L. (Bonos et al. 2002).

1.2 Three Major Bentgrass Species

1.2.1 Creeping Bentgrass (*A. stolonifera* L.)

Creeping bentgrass is a cool-season grass species that is native to Western Europe. It is not only adapted to cool and humid areas but also preferred to be used in warmer places at golf courses due to its fine texture. Creeping bentgrass has high density and mowing height as low as 3 mm; these features make it suitable to be used especially in greens though it is also used in tees and fairways of the golf courses (Warnke 2003). It requires high maintenance, so it is not ideal for home

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lawns. It has low growth habit and aggressive spreading (Casler 2006).

Creeping bentgrass is commonly referred to as *A. stolonifera* L., but other synonyms are also used such as *Agrostis palustris* Huds. and *A. stolonifera* L. var. *palustris* (Huds.). It is a strict allotetraploid with a genome $A_2A_2A_3A_3$ ($2n = 4x = 28$) (Warnke 2003). Complete chloroplast genome sequence of *A. stolonifera* (136,584 bp) was presented and compared with other grass species. The chloroplast genome contains 53.6% coding regions (44.7% protein coding genes and 8.9% RNA genes), and the rest is noncoding regions (Saski et al. 2007).

Creeping bentgrass requires vernalization, and flowering occurs generally in late spring to early summer (Warnke 2003). Interspecific hybridization of creeping bentgrass is possible with five different *Agrostis* species (*A. canina* L., *A. castellana* Boiss. & Reut., *A. gigantea* Roth, *A. capillaris* L., and *A. vinealis* Schreb).

Due to its stoloniferous growth, thick thatch layers can be formed, if it is not managed properly. This then serves as a good environment for pathogens and insects. It is susceptible to many diseases, but dollar spot (*Sclerotinia homeocarpa*), brown patch (*Rhizoctonia solani*), *Typhula* blight (*Typhula incarnata* or *Typhula ishikariensis*), the so-called gray snow mold, and *Fusarium* blight (*Fusarium roseum* and *Fusarium tricinctum*), also named as pink snow mold, are the main concerns.

A study with rhizobacteria, isolated from roots of bentgrass and bermudagrass located from USGA golf putting greens, showed that *Pseudomonas* was the dominant genus in the roots of bentgrass for denitrification. For both species, 17% of the isolates were identified by GC-FAME (gas chromatography – fatty acid methyl ester) and 16S rDNA analyses. Besides, nitrous oxide reductase (*nosZ*) and nitrite reductase genes (*nirK* and *nirS*) were recognized (Wang and Skipper 2004).

1.2.2 Colonial Bentgrass (*A. capillaris* L.)

Colonial bentgrass is both referred as *A. capillaris* L. and *Agrostis tenuis* Sibth. Origin of colonial bentgrass is Europe and temperate Asia. It is a cool-

season grass and also named as brown top. While it is used in lawns and sports fields such as tennis courts and golf courses, it is also a choice for erosion control (Hubbard 1984). It is compatible with other species (Casler 2006).

Colonial bentgrass is preferred on fairways and tees at golf courses with a mowing height of 1.0–2.5 cm; hence, it is not much suitable for greens. It has lower maintenance needs than creeping bentgrass; instead it has more limitations (Ruemmele 2003). Colonial bentgrass can adapt to different environments with forming specific ecotypes (Busey 2003).

The colonial bentgrass (*A. capillaris* L.) with the genome $A_1A_1A_2A_2$ and creeping bentgrass (*A. stolonifera* L.) with the genome $A_2A_2A_3A_3$ are both allotetraploid and have 14 chromosome pairs ($2n = 4x = 28$) (Ruemmele 2003; Warnke 2003). Since they are sexually compatible, hybrids can be produced.

Genomics studies facilitate analysis of many species for their origin and evolution with the use of many tools it provides. Expressed sequence tag (EST) sequence analysis of colonial and creeping bentgrass A_2 genomes suggested that these genomes diverged from a common ancestor at about 2.2 million years ago and supported the previous studies that A_2 genomes were common for both species. Their findings also showed that both creeping and colonial bentgrasses were closer to tribe Poeae than tribe Aveneae that are actually closely related tribes (Rotter et al. 2007).

1.2.3 Velvet Bentgrass (*A. canina* L.)

Velvet bentgrass is referred as *A. canina* L., which is native to Europe. It is diploid ($2n = 2x = 14$) with genome designation of A_1A_1 . It is confused widely with *A. vinealis* ($2n = 4x = 28$) which is an autotetraploid of *A. canina*.

Velvet bentgrass is a cool-season grass with very fine texture, maintaining a good putting surface at golf courses, and it has stoloniferous growth habit. It is the most shade-tolerant among other *Agrostis* spp. It has good heat, low temperature, and drought tolerance. Besides, it is more tolerant to acidic soils than all other bentgrasses. However, it needs high maintenance (Brilman 2003) and it has low growth habit (Casler 2006).

1.3 Three Minor Bentgrass Species

1.3.1 Redtop (*A. gigantea* Roth)

Redtop is a cool-season, perennial grass, which is referred as *A. gigantea* Roth or *A. alba*. Redtop bentgrass (*A. gigantea* Roth) is allopolyploid with the genome $A_1A_1A_2A_2A_3A_3$ ($2n = 6x = 42$), which are the inclusion of the genomes of both creeping and colonial bentgrasses. It can be crossed with creeping bentgrass to produce infertile hybrids.

Redtop is used as forage or turf or for reclamation purposes for revegetation of disturbed areas. It has low maintenance (Brede and Sellmann 2003). It has rapid establishment (Casler 2006). It is tolerant to high metal concentrations and is the most salt-tolerant among other bentgrasses. It can be used in breeding programs for enhanced tolerance. It has the potential to be improved as forage or turf (Brede and Sellmann 2003).

1.3.2 Highland Bentgrass (*A. castellana* Boiss. and Reuter)

Highland bentgrass is a dense turf that has good color in winter times. It is tetraploid, but its hexaploid types can also be found. It is adapted to warmer and less humid areas than *A. capillaris*. *A. castellana* has mowing heights ranging from 1.0 to 1.9 cm and can be used for turf purposes. But it is better if used as a low maintenance grass or for reclamation purposes (Brede and Sellmann 2003); it is a drought-tolerant species (Casler 2006).

1.3.3 Idaho Bentgrass (*Agrostis idahoensis* Nash)

Idaho bentgrass is a cool-season, perennial grass species, which is native to North America. Depending on its mowing height, it shows more tolerance to diseases than any other bentgrass types. It can be used for improvement of *Agrostis* germplasm in pest resistance (Brede and Sellmann 2003).

1.4 Marker Systems

The origin of colonial bentgrass is temperate Asia and Europe. It is well adapted to low temperatures and partial shade and mostly used for tees and fairways of the golf courses. In order to study genetic variation and relation among colonial bentgrass populations, random amplified polymorphic DNA (RAPD) marker analysis was applied, revealing high degree of genetic diversity. The accessions introduced from Europe to USA had genetic resemblance with the European cultivars; however, accessions from Bulgaria and Turkey showed the lowest genetic similarity, though these two regions are not geographically distant from each other (Rajasekar et al. 2007).

Twenty-two different colonial bentgrasses (*A. capillaris* L.) from different countries, including few commercial cultivars, were analyzed with 128 amplified fragment length polymorphism (AFLP) markers, resulting in high level of genetic biodiversity. Diverse gene pool availability was especially investigated in the accession rather than the commercial cultivars (Zhao et al. 2006). It is natural that during domestication events, through breeding, cultivated species loses its genetic diversity and gains uniformity. So that wild relatives or other species play an important role in plant improvement programs. Colonial bentgrass is a good source with its diverse germplasm for the improvement of other *Agrostis* species or turfgrass types.

Creeping bentgrass (*A. stolonifera* L.), colonial bentgrass (*A. capillaris* L.), and velvet bentgrass (*A. canina* L.) are the extensively used bentgrasses (*Agrostis* spp.) on putting greens, tees, and fairways of the golf courses at temperate regions. They are highly difficult to be identified using their morphological features. However, it was investigated that RAPD markers were effective in differentiation and identification of bentgrass species at molecular level (Hollman et al. 2005). Besides the use of RAPD markers, sequence characterized amplified region (SCAR) markers were designed for colonial and creeping bentgrasses. They were found to be effective to differentiate between these two bentgrasses and these from other *Agrostis* spp. This technology was also recommended to be used for selecting the progenies produced from interspecific hybridization between colonial and creeping bentgrasses (Scheef et al. 2003).

Few creeping bentgrass cultivars were identified using restriction fragment length polymorphism (RFLP) markers (Caceres et al. 2000), which is one of the first marker types to be used in linkage studies and an important tool for genetic mapping. Moreover, molecular marker-based linkage map was developed for creeping bentgrass using AFLP, RAPD, and cDNA-RFLP markers. Further use of this map would be useful in detection of quantitative trait loci (QTL) and marker-assisted breeding of agronomically important traits like disease resistance (Chakraborty et al. 2005). Transcript polymorphism analysis using SRAP system in three different bentgrasses (colonial, creeping, and velvet bentgrass) was the first study to be reported on turfgrasses. The ESTs obtained from this study could be used in turfgrass improvement programs (Dinler and Budak 2008).

AFLP analysis for tetraploid creeping bentgrass and hexaploid redtop bentgrass was performed to investigate genetic diversity of the old and new cultivars, European plant introductions, and gray snow mold (*T. incarnata* Lasch) resistant genotypes of creeping bentgrass and could be used in plant improvements and gene mapping studies (Vergara and Bughrara 2004). For dollar spot disease, QTL analysis was performed to deeply understand the genetics of the resistance to this disease. It was found that dollar spot resistance is a quantitative trait and highly heritable. RAPD markers tightly linked to this QTL could be a useful tool in breeding programs of *Agrostis* spp. (Chakraborty et al. 2006).

1.5 Heat Stress and Drought Tolerance

Plants are affected from temperature changes since all metabolic activities take place at a certain temperature. Heat stress tolerance level differs according to the plant species. Some plants can become tolerant to high levels of heat stress by time, and that can be exhibited as a genetic character.

In cool-season grass species, heat stress causes leaf senescence that is regulated by cytokinins. The decrease in cytokinin content causing an increase in leaf senescence process was reported in *Agrostis* species (Xu and Huang 2007). Chlorosis followed by leaf senescence causes a decline in chlorophyll content and photosynthetic capacity resulting in reduced plant

growth (John et al. 1995) and, especially for turfgrass, loss of deep green color, which is an important parameter for its economic value. *SAG12-ipt* (ligation of *ipt*, adenine isopentenyl transferase gene to *SAG12*, senescence-activated promoter) gene integration into creeping bentgrass resulted in transgenic lines that had faster growth under normal temperatures and increased *ipt* gene expression and cytokinin production suppressed heat-induced leaf senescence (Xu et al. 2009) resulting in an improvement for cool-season grasses exposed to heat stress.

The main problem of growing cool-season grasses in temperate areas is the increasing temperature in summer time causing a decline in plant productivity. Using differential display analysis, upregulated genes (18 in *A. stolonifera* and 22 in *A. scabra*) in response to heat stress were identified and most genes were found in both species. *A. scabra* is a heat-tolerant species for which the significant decline in soluble protein content was observed at 40°C, whereas *A. stolonifera* is nontolerant under heat stress, and protein content conservation was up to 30°C (Xu and Huang 2008a, b). Only three of these genes were expressed in *A. scabra*, and it was reported that these genes might have been serving for the heat tolerance. Previous analysis of thermal and nonthermal *A. scabra* with 60 RAPD loci showed that they were not clustered independently but they were distantly related although they were morphologically similar (Tercek et al. 2003). If it is the case, this might enlighten the mechanism of plants' tolerance to heat stress and facilitate development of in heat tolerant cultivars (Xu et al. 2008).

Heat shock proteins (HSPs) in plants are synthesized in response to heat stress for protection. The small HSPs that are localized in chloroplasts (CP-sHSPs) are essential in heat tolerance expressing varying levels of its isoforms. Two additional isoforms of CP-sHSPs in heat-tolerant variants of creeping bentgrass (Penn-cross cultivar), which were generated under heat stress and selected for heat tolerance, were accumulated with respect to heat-sensitive variants that were not subjected to heat stress. These isoforms were genetically linked to heat tolerance (Park et al. 1996; Luthe et al. 2000). The study of CP-sHSP encoding genes isolated from heat-tolerant and heat-sensitive variants showed that the differences and variation in the expression of CP-sHSPs were related with the environmental adaptation and suggested that the amount of CP-sHSPs

might have been the source of heat tolerance (Wang et al. 2003; Wang and Luthe 2003).

During the growth of creeping bentgrass species, the temperature was increased gradually. Following heat acclimation, the creeping bentgrass showed an improved tolerance under heat stress. In consecutive studies, it was found that heat acclimation was associated with suppressed lipid peroxidation (Larkindale and Huang 2004), enhanced heat shock protein expression (He et al. 2005), and enhanced photosynthetic activity by higher pigment content and rubisco activity (Liu and Huang 2008).

A. scabra is a thermal species found to be tolerant to high temperatures like 40–45°C and to be living at the Yellowstone Natural Park (Tercek et al. 2003). For heat tolerance capacity, cool-season, perennial grasses, *A. scabra* with two genotypes and commercially important *A. stolonifera* with ten genotypes were investigated for the expression of *AsEXPI* gene under heat stress. Heat tolerance of the treated plants differed according to the *Agrostis* species and genotypes and fell into three classes as being most tolerant to most sensitive. Heat tolerance level was positively correlated with the level of *AsEXPI* gene expression. Totally, four genotypes, two from *A. scabra* and two from *A. stolonifera*, exhibited the highest level of gene expression with response to heat stress, being the most heat-tolerant ecotypes. This expansin gene, *AsEXPI*, was found to be highly upregulated in shoots and affected the heat tolerance of both species as “whole-plant.” The identification of this gene as response to heat tolerance in C3 *Agrostis* species for the first time was suggested to be important both for the study and investigation of the heat tolerant germplasm of grasses (Xu et al. 2007). In a further study, novel heat responsive genes were identified in *A. scabra* by subtractive suppression hybridization approach. The differentially expressed genes were classified based on their role in stress and defense mechanism, signaling and transcription, and protein or carbon metabolism (Tian et al. 2009).

For plants, the soil temperature is more important than temperature of the air. The soil temperature primarily affects the roots. Heat-tolerant *A. scabra* is adapted to high soil temperatures at geothermal locations, whereas heat-sensitive *A. stolonifera*, adapted to cool climatic regions, cannot survive at high temperatures. When compared to *A. stolonifera*, whole-plant carbon balance and root carbon utilization was maintained positively,

and the root respiration rate was low or downregulated in *A. scabra* at high temperatures. Both factors were suggested to be the reason for root thermotolerance adaptation of cool-season grasses (Lyons et al. 2007). Both short-term and long-term respiratory acclimation was investigated to be accompanied with root thermotolerance *A. scabra* at increasing temperatures. *A. scabra* was found to be giving less response, in terms of root respiration, than *A. stolonifera*. The adjustment of root carbon utilization was stated to be caused by respiratory acclimation that the need for increased respiratory energy was lowered at high temperatures, resulting in increased root survival (Rachmilevitch et al. 2008).

Thermal *A. scabra* has higher root viability than heat-sensitive *A. stolonifera*. The protein and phosphoprotein patterns of *A. scabra* and *A. stolonifera* were mainly different under heat stress. It was proposed that sucrose synthase, glutathione S-transferase, superoxide dismutase, stress-inducible heat shock protein, and aldolase phosphorylation might have been associated with root thermotolerance in cool-season grasses under heat stress (Xu and Huang 2008a, b).

Since carbon utilization and accumulation are thought to have a role in heat tolerance and enhancement of plant survival in perennial grass species, three species of cool-season turfgrasses, colonial bentgrass (*A. capillaris* L.), creeping bentgrass (*A. stolonifera* L.), and velvet bentgrass (*A. canina* L.), were examined based on their shoot and root carbon partitioning and carbohydrate accumulation. Measuring the turf quality and relative leaf water content revealed that velvet bentgrass was the most tolerant under drought conditions and colonial bentgrass had the most amendatory potential. According to total nonstructural carbohydrate content measurements, increased carbon accumulation in roots after initial drought application and then its gathering in stems and leaves at increased drought durations suggested an adaptive response for drought survival (DaCosta and Huang 2006a, b, c). Other study with creeping bentgrass with different irrigation applications resulted in enhanced carbohydrate levels in leaves and roots when irrigating at wilt (Fu and Dernoeden 2008).

Velvet bentgrass was found to be the most drought-tolerant bentgrass species among others. In colonial, creeping, and velvet bentgrasses, prolonged drought stress caused a decrease in antioxidant enzyme activities and an increase in lipid peroxidation. However, in velvet bentgrass, oxidative damage was prevented for