

# Exploiting GENETIC DIVERSITY among unadapted WHEAT LANDRACES from different geographical origins revealed by SSRs

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## Introduction

Over several thousand years, landraces of hexaploid wheat have developed under a variety of different edaphic and climatic environments. This has led to the evolution of a large number of ecotypes adapted to specific local environments, which are certainly an important source of genetic variation in wheat. These ecotypes can be differentiated using SSR markers, and the resulting dendrograms can be used to reconstruct the similarities between landraces, and patterns of introduction and spread of wheat around the world.

## Materials and Methods

In this study, 30 SSR markers covering the A, B, and D genomes were used to characterize 478 hexaploid wheat landraces collected from 51 different countries as part of a large scale genotyping project within the Generation Challenge Program (<http://www.generationcp.org/index.php>). SSR marker data is available on the GCP website, <http://gcp.cgrinfo.net/>. Genomic DNA was extracted from bulked leaves harvested from 7–10 young plants using a CTAB procedure reported in (<http://www.cimmyt.cgiar.org/ABC/Protocols/manualABC.html>). Information about each SSR marker is available at: [http://www.cimmyt.org/english/docs/manual/dbases/fingerprint\\_instructions\\_manual.htm](http://www.cimmyt.org/english/docs/manual/dbases/fingerprint_instructions_manual.htm). Primers were labeled with fluorescent dyes and PCR products were multiloaded onto an ABI Prism 377 automatic DNA Sequencer (Perkin Elmer/Applied Biosystems). Average number of alleles per locus (NA), polymorphic information content (PIC), heterozygosity (H) and total gene diversity (HT) were calculated within and between groups. Genetic distances among pairs of genotypes were calculated using Modified Rogers distance (MRD) using the statistical software R. Genetic distance values were used to generate an unweighted pair-group clustering based on the arithmetic averages (UPGMA) phenogram.

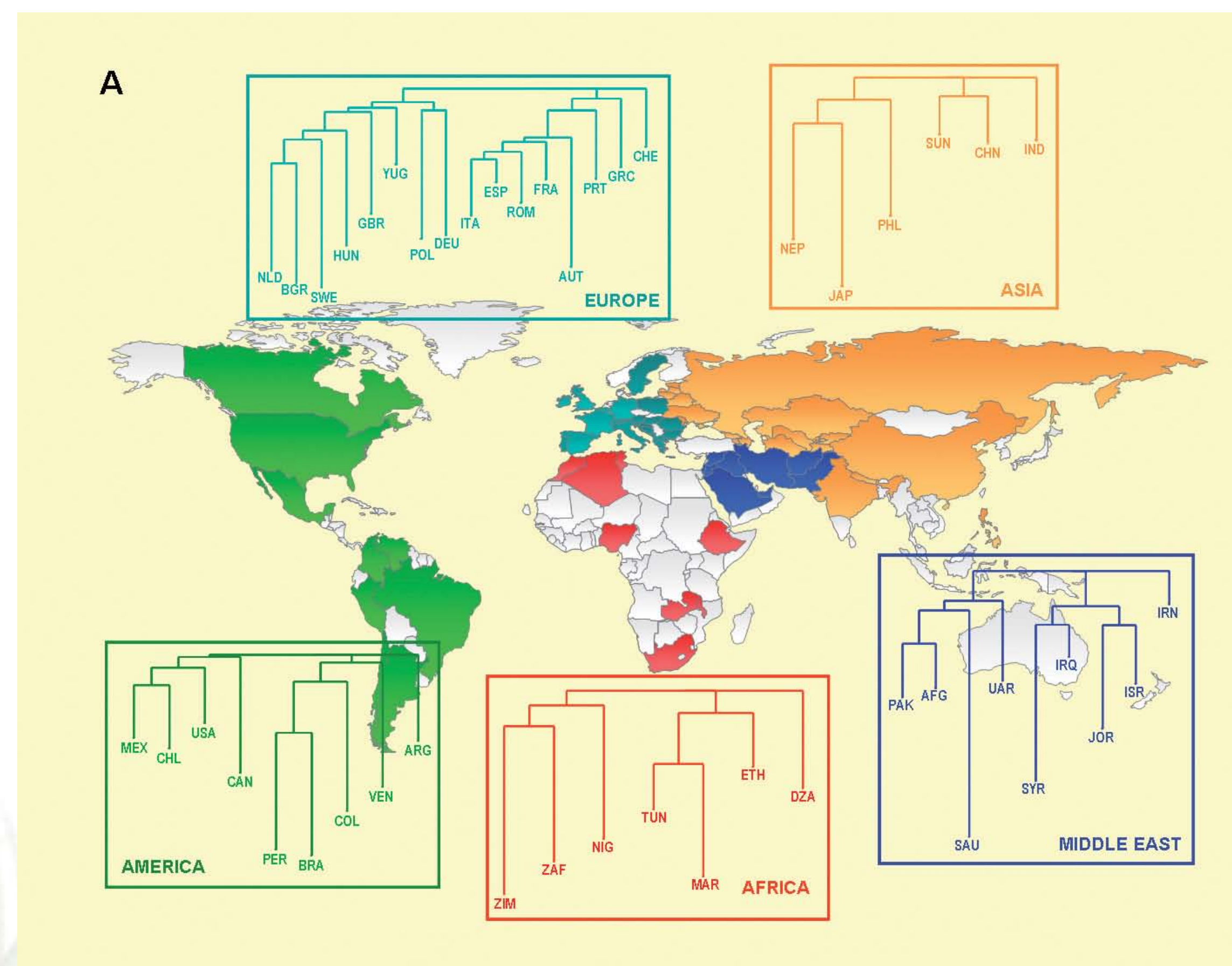
## Results and Discussion

Substantial levels of genetic variation were found in the selected materials. The average number of alleles per locus across the entire set of landraces was 13.7 and ranged from 3 to 25. The highest values of average number of alleles per locus and gene diversity were observed in landraces collected in China-Japan, the former Soviet Union, and Saudi Arabia-United Arab Emirates (Table 1). The lowest values were observed in landraces collected in Brazil-Venezuela and Tunisia-Morocco. The frequency of unique alleles in a certain country ranged from 0.009 to 0.25 (data not shown). The highest numbers of unique alleles were observed in China-Japan and Ethiopia. When grouping the landraces regarding their continent or region origin, highest values of the average number of alleles per locus were observed in landrace from countries in the Middle East and Asia, whereas lowest values were observed in landraces from countries in North America and Oceania (Table 1). The highest number of unique alleles was found in Asia.

Thousands of years after domestication and expansion into new growing areas, wheat culture and migration from the Fertile Crescent to Europe, Africa and Asia, as evidenced from archaeological finds, can be confirmed with the SSR markers. Neighbor joining clustering showed a clear separation between the European and the Asian wheat germplasm (Fig 1B). The germplasm from European countries forms two sub-clusters (North-western, and Central Europe and the Mediterranean) which could reflect the oldest migration pathways of the Neolithic farmers bringing initial wheat germplasm soon after 6000 BC from the center of domestication (Fig. 1A). The first pathway followed the Mediterranean coast to Spain, the second passed northwards and traversed Hungary, Poland and Germany, reaching the North Sea before 4000BC (Harlan, 1981; Bonjean et al., 2001). The joint cluster of wheat landraces from South Asia, the former Soviet Union and China could reflect the two most important routes of wheat in to China; the Myanmar route which started from Afghanistan through the Asian continent (Fig. 1B) (Harlan, 1981). The relative position of the USA, Canada, and Australia– New Zealand landraces clearly confirms the European origin of North American and Oceanic wheat, which were introduced there during the sixteenth and the nineteenth centuries, respectively (Fig 1B) (Harlan, 1981). Landraces from Central and South America cluster into the same group although main introductions are reported by the Spaniards starting at ca. 1520. The location of southern African landraces within European and Asian germplasm clusters confirms the establishment of wheat by early settlers from Europe and the East Indies (Fig 1B) (Bonjean et al., 2001).

**Table 1. Number of landraces (N), average number of alleles per locus (N<sub>A</sub>), number of unique alleles (N<sub>U</sub>), gene diversity (H<sub>T</sub>), and inbreeding coefficient (f) calculated from 478 hexaploid wheat landraces of 51 countries and 7 continents or regions genotyped with 30 SSR markers.**

Countries	N	N <sub>A</sub>	N <sub>U</sub>	H <sub>T</sub>	f
AFG	36	5.7	3	0.558	0.83
ARG	6	2.9	1	0.483	0.80
AUS	8	3.4	0	0.545	0.83
BGR-ROM	9	3.3	0	0.497	0.80
BRA-VEN	4	2.2	0	0.406	0.71
CHE-AUT	8	3.5	1	0.543	0.86
CHL-COL-PER	8	3.4	2	0.526	0.80
CHN-JPN	32	6.8	11	0.623	0.81
DEU-NLD	3	2.4	0	0.463	0.80
DZA-NIG	4	2.5	0	0.471	0.80
ESP	22	5.0	2	0.528	0.82
ETH	26	5.6	12	0.582	0.87
FRA	18	4.7	4	0.564	0.89
GBR-SWE	3	2.2	0	0.428	0.86
GRC-CYP	9	4.0	1	0.586	0.83
HUN-POL	4	2.8	2	0.501	0.86
IND-NEP-PHL	25	6.0	5	0.608	0.83
IRN	14	5.0	2	0.601	0.83
IRQ	7	3.2	0	0.492	0.90
ISR-JOR-SYR	15	4.0	0	0.507	0.84
ITA	20	3.9	4	0.457	0.82
MEX	7	3.4	0	0.535	0.69
NZL	4	2.8	0	0.514	0.75
PAK	40	5.9	9	0.526	0.73
PRT	10	4.1	2	0.596	0.83
SUN	31	6.6	6	0.623	0.82
TUN-MAR	10	3.2	1	0.425	0.85
TUR	58	6.5	10	0.594	0.82
UAR-SAU	9	4.2	2	0.615	0.83
USA-CAN	12	4.1	0	0.545	0.78
YUG	12	4.7	2	0.584	0.80
ZAF-ZIM	4	2.8	0	0.513	0.86
AFRICA	44	7.3	11	0.630	0.87
ASIA	89	10.0	59	0.656	0.83
EUROPE	114	9.4	11	0.647	0.85
MIDDLE EAST	183	10.3	21	0.656	0.83
NORTH-AMERICA	18	4.8	0	0.586	0.75
OCEANIA	11	3.9	0	0.576	0.81
SOUTH AMERICA	19	5.4	3	0.629	0.81



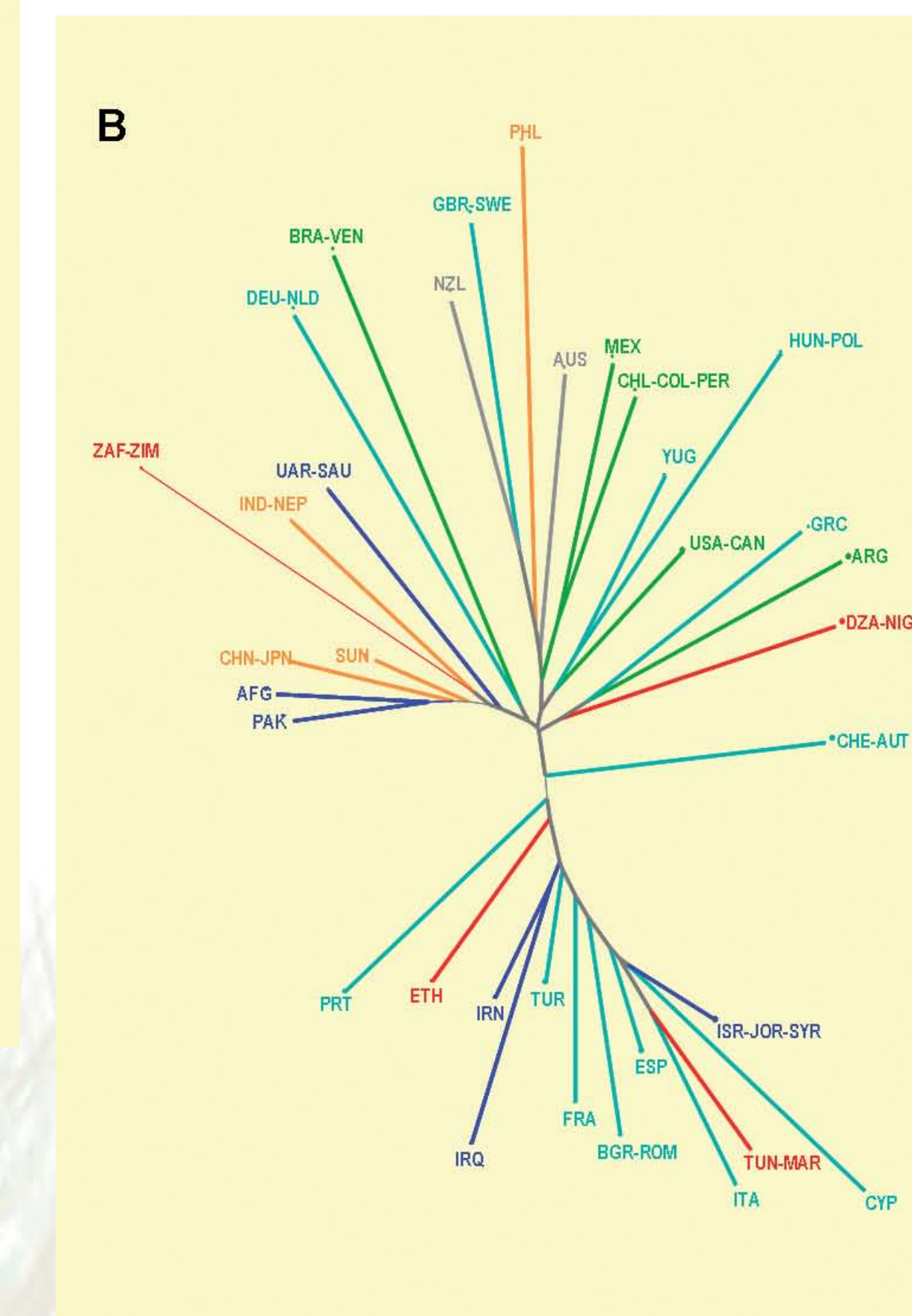
## Conclusions

SSR markers are useful for determining both recent paternity/identity, as well as ancient relationships. This information may be used to determine the potential utility of different landraces for broadening the germplasm base for any given region, seeking new traits or alleles of interest, and aid in plant genetic resource collection and conservation.

## References

Harlan JR, (1981) The early history of wheat: earliest traces to the sack of Rome. In: Evans LT, Peacock WJ (eds.) *Wheat Science – Today and Tomorrow*, Cambridge University Press, Cambridge, pp 1-19.  
Bonjean, AP, Agnus WJ. 2001 *The World Wheat Book, A history of Wheat Breeding*. Lavoisier Publishing, Paris, France.

**Fig 1. Dendrograms of 487 hexaploid wheat landraces grouped in to 51 different countries based on neighbor joining clustering of Rogers distance calculated from 30 SSR markers. A) Dendrograms of countries from five defined regions, B) Dendrogram of all considered countries. Names refer to the UN/IOC three letter country abbreviations at (see Appendix).**



## Appendix:

AFG=Afghanistan, MAR=Morocco,  
ARG=Argentina, MEX=Mexico,  
AUT=Austria, NEP=Nepal,  
BRA=Brazil, NIG=Nigeria,  
BGR=Bulgaria, NLD=Netherlands,  
CAN=Canada, PAK=Pakistan,  
CHE=Switzerland, PER=Peru,  
CHL=Chile, PHL=Philippines,  
CHN=China, POL=Poland,  
COL=Colombia, PRT=Portugal,  
DEU=Germany, ROM=Romania,  
DZA=Algeria, SAU=Saudi Arabia,  
ESP=Spain, SUN=former Soviet Union,  
ETA=Ethiopia, SWE=Sweden,  
GBR=Great Britain, TUN=Tunisia,  
GRC=Greece, UAR=United Arab Emirates,  
HUN=Hungary, USA=United States of America,  
IND=India, VEN=Venezuela,  
IRN=Iran, YUG=former Yugoslavia,  
IRQ=Iraq, ZAF=South Africa,  
ISR=Israel, ZIM=Zimbabwe,  
ITA=Italy,  
JAP=Japan,  
JOR=Jordanian,