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*Communication*

# Digital Phenotyping for Next-Generation Research on Plant Genetic Resources: The Case of RDA-Genebank

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**Abstract:** The National Agrobiodiversity Center under the Rural Development Administration (RDA) in Jeonju, Republic of Korea, is the largest international genebank of Korea. The National Agrobiodiversity Center has continuously been making efforts to introduce overseas genetic resources to its collection and has currently grown into a world-class PGR holding genebank. Currently, several steps are undertaken to, improve the accessibility of the collection to national as well as international researchers, improve data available on the resources and amend the passport information of the accessions. With the implementation of the Nagoya Protocol, the origin of genetic resources is being highlighted as an important input in the passport information. The RDA-Genebank actively responds to the Nagoya Protocol by supplementing passport data of resources lacking information on the origin. In addition, a large number of conserved resources are continuously multiplied, and agronomic traits are investigated concurrently. With the traditional methods of characterization of the germplasm requiring a significant amount of time and effort, we have intiated high-throughput phenotyping using digital techniques to improve our germplasm data. Primarily, we have started adding seed phenotype information followed by measuring root phenotypes which are stored under agronomic traits. This may be the initial step to use large-scale high-throughput techniques for a germplasm. In this communication, we aim to provide an introduction to the RDA- Genebank, adopted international standards, and establishment of high throughput phenotyping techniques for improvement of passport information.

**Keywords:** genebank; digital phenotyping; RDA-Genebank; Nagoya Protocol

## 1. Introduction to the National Agrobiodiversity Center (RDA-Genebank)

The National The National Agrobiodiversity Center in Jeonju, Republic of Korea, under the administration of the Rural Development Administration (RDA), plays a pivotal role in the collection, evaluation, conservation, and distribution of diverse plant genetic resources, including both seeds and vegetative germplasm. With the establishment of a low-temperature seed storage facility in 1975 and a seed management office in 1976, around 3,300 germplasm were managed at local agricultural institutions. Later in 1980, official documentation of 12,865 rice germplasm conserved by local agricultural institutions and universities was carried out, followed by their addition to the low-temperature seed storage collection. While seed germplasm were conserved at the genebank, external management institutions managed vegetative germplasm. In 1993, RDA-Genebank began international cooperation starting with the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) (1993–2019). At RDA-Genebank, researchers aim to secure germplasm diversity by continuously collaborating with international joint research institutes including Uzbek research institute of plant industry (UzRIPI) in Uzbekistan (1996–2004), department of agriculture (DOA) genebank in Thailand (1998), Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (1998–1999) in Germany, International Rice Research Institute (IRRI) in the Philippines, PASRI in Mongolia,

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(National Bureau of Plant Genetic Resources (NBPGR) in India, and GAAS (Georgian Academy of Agricultural Sciences) in Georgia. Adoption of international policies such as the Convention on Biological Diversity (CBD) at the United Nations conference on environment and development (UNCED) in 1992 and the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization in 2010 has further reinforced germplasm conservation efforts. Despite the limitations on data disclosure and international distribution of germplasm, Korea remains one of the significant countries conserving plant biodiversity (Figure 1).

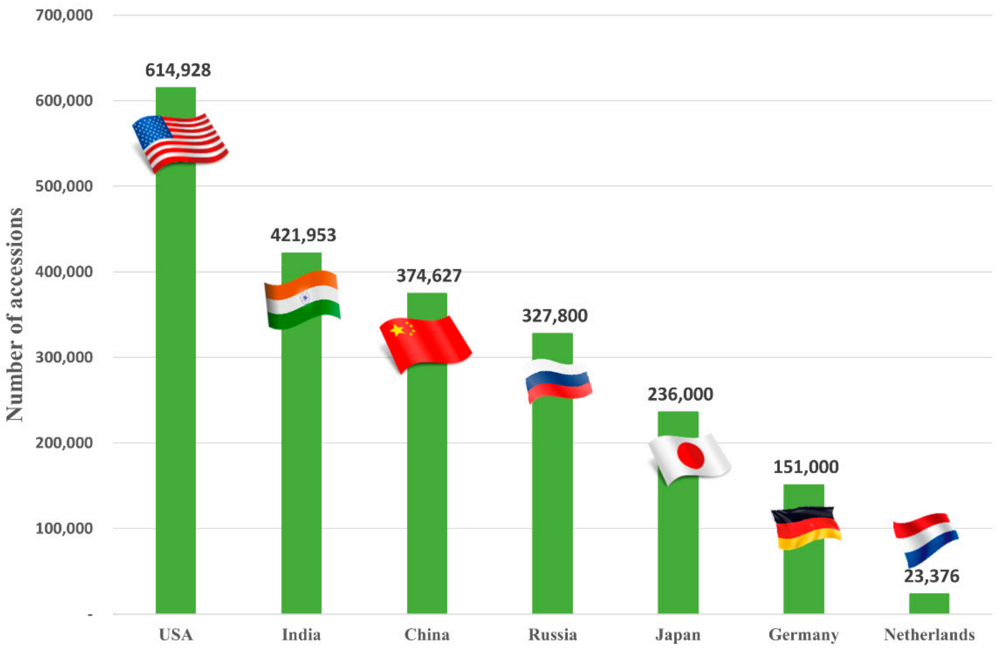

**Figure 1.** Country-wise statistics of the number of germplasm conserved at their international genebanks across the world[1–6].

## 2. RDA-Genebank`s Efforts on the Nagoya Protocol

The Nagoya Protocol is an international agreement that aims to promote the fair and equitable sharing of benefits arising from the use of genetic resources, including those held in genebanks. The protocol was adopted in 2010 under the United Nations Convention on Biological Diversity (CBD) and entered into force in 2014. The history of the Nagoya Protocol can be traced back to the CBD, which was adopted at the 1992 Earth Summit in Rio de Janeiro, Brazil. The CBD recognized the importance of conserving biodiversity and the need to ensure the fair and equitable sharing of benefits arising from the use of genetic resources. However, it was found that the existing legal framework did not adequately address the issue of benefit-sharing. This led to the development of the Nagoya Protocol, which provides a framework for the fair and equitable sharing of benefits arising from the utilization of genetic resources. The protocol establishes rules for access to genetic resources, including prior informed consent and mutually agreed terms, and requires users to share the benefits arising from the use of those resources with the providers. In the context of genebanks, the Nagoya Protocol has important implications for the collection and utilization of genetic resources. Genebanks are required to comply with the access and benefit-sharing requirements of the protocol when obtaining new genetic resources, and must also ensure that any benefits arising from the use of those resources are shared fairly and equitably with the countries and communities that provided them.

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Overall, the Nagoya Protocol represents a significant milestone in the international effort to promote the conservation and sustainable use of genetic resources, including those held in genebanks, and to ensure that the benefits of these resources are shared fairly and equitably. In accordance with the Nagoya Protocol, RDA-Genebank continuously updates passport data on the origin of germplasm to actively respond to the utilization of genetic resources and the sharing of benefits accordingly. Of the 272,351 accessions conserved in the RDA-Genebank, 42,698 (15.7%) accessions need to be supplemented with origin information. As a method of supplementing origin information, the passport data disclosed on the homepage of the relevant genebank was reflected based on the original international genebank resource number. As a result, 7,773 accessions (17.3%) out of 42,698 accessions supplemented with origin information.

## 3. RDA-Genebank`s Distribution of Germplasm

Over the past two decades, the RDA-Genebank has distributed an impressive 770,280 accessions, at an annual rate of 38,514 accessions, on average. Nearly all of these accessions (99.27%) were domestically distributed in Repubic of Korea, while only a minor proportion (0.73%) was circulated internationally. The trend of domestic distribution has remained persistently upwards, with a minor downward fluctuation in 2008 that may be attributed to the global economic crisis. However, the subsequent year witnessed a substantial rise in distribution, potentially attributable to the base effect (Figure 2). In 2018, the RDA-Genebank initiated a collaboration research program, the Technology Development Project for Customized Agricultural, Forestry, and Fishery Products and Services, partnering with agricultural organizations such as seed companies and private research institutions. The objectives of this program were to broaden and improve the characterization and evaluation information of the conserved germplasm, augment its diversity, propagate it, and concurrently conduct characterization research. Through chemical compositon profiling or disease resistance evaluation, commendable genetic resources have been shortlisted from the outcomes of these research endeavors, to be recommended for breeding and further scientific investigations. While the RDA-Genebank disperses germplasm internationally, the predominant proportion of the disseminated germplasm has been channeled towards international cooperative projects under the aegis of the RDA. The RDA-Genebank also distributes germplasm overseas, but the vast majority of the distributed germplasm were for international cooperation projects at RDA. During 2010-2013, a total of 1,343 accessionswere distributed for local propagation of rice to the IRRI, The Philippines. For the purpose of germplasm exchange with international genebanks, RDA-Genebank has introduced 568 accessions in ARS (2012) in the US, 995 accessions at UzRIPI in Uzbekistan (2011~2014), 10 in VIR in Russia (2014), 235 in AVRDC in Taiwan (2013~16), and 232 in GAAS in Georgia (2015~ 2016) as a part of its germplasm exchange policy. We have also supplied to private entities such as distribution of 62 sesame accessions to Tainan District Agricultural Reasearch and Extension Station in Taiwan. The distribution of overseas can be categorized into two types: those sent through KOPIA center as part of ODA projects that provide country-specific farming techniques, and those distributed through the 3-FACI (AFACI, KAKACI and KoLFACI) agreement for multi-continental research aimed at solving common farming problems. Despite RDA-Genebank's possession of world-class germplasm, limited overseas distribution has occurred due to the unavailability of individual resource information on online platforms such as GENESYS, managed by the International Treaty on Plant Genetic Resources for Food and Agriculture. Exceptions to this pattern have only happened when researchers with Korean affiliations have utilized publicly available history information to apply for resource distribution.

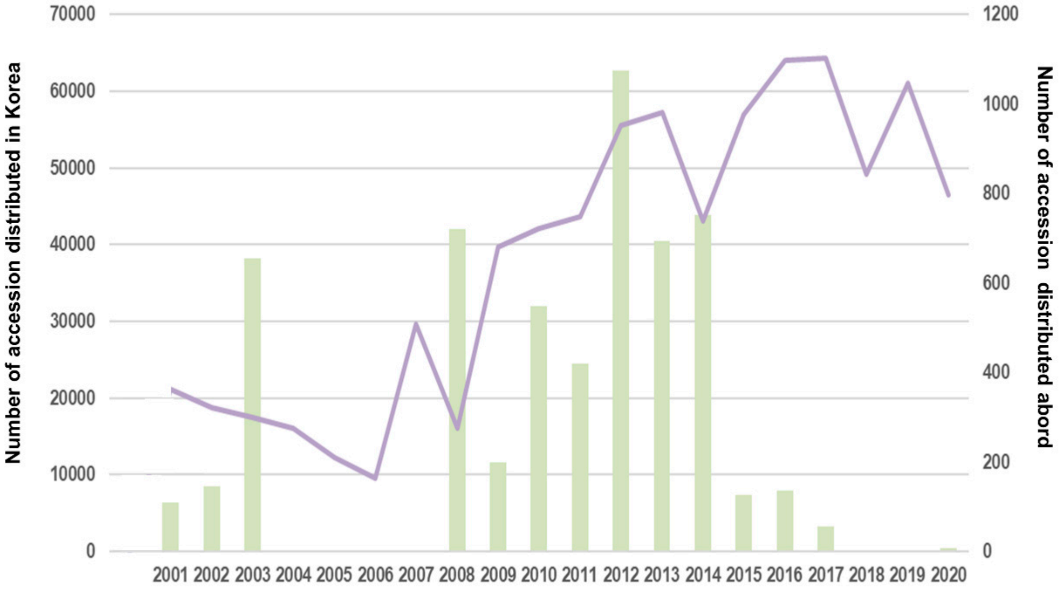

**Figure 2.** Statistics of germplasm dissemination by RDA-Genebank in the past 20 years from 2001 to 2020. The purple trendline shows germplasm distributed domestically (Korea) and the green columns shows germplasm distributed abroad.

## 4. Agronomic Traits and Passport Data

In order to improve the utilization of conserved germplasm, systematic passport data management is indispensable [7]. Germplasm deposited in RDA-Genebank are stored in cold storage after verification of the seed viability. Users of the genebank who include breeder and researchers are provided with technical support to select germplasm based on passport data to use appropriate germplasm for their purpose. Germplasm deposited through domestic and overseas field collections and institutional introductions are managed by initially assigning temporary numbers in RDA-Genebank. For domestic and overseas field collected germplasm, the passport data prepared by the collector is adopted, and for the introduction of germplasm held by domestic and foreign institutions, a database is prepared by basically reflecting the passport data held by the existing institutions. In the period between 1985-2004, a database was established including 14 descriptors, including crop type (food, horticulture and specialty crop, etc), origin, scientific name, germplasm name, and statues (cultivar, landrace, and wild**,** etc) for the passport data of 150,000 accessions collected and introduced to the RDA-Genebank. In order to manage temporary germplasm as nationally registered resources, the number of seeds and viability standards established for each crop must be met. Through the periodic propagation project, the viability and seed count are updated, and characterization data for each crop is created according to the standards of RDA-Genebank. In response to consumer preferences, there is a notable increase in the incorporation of evaluation data concerning chemical composition profiles, such as glucosinolate levels in Brassica ssp, and disease resistance. This highlights the importance of considering these factors in the evaluation process. Since the 1990s, RDA-Genebank has systematically investigated its conserved genetic resources for their qualitative and quantitative traits to promote the use of germplasm by consumers. During the early stages, the characterization centered on seed viability and primary characteristics of germplasm such as seed coat color, width and length. From 1995, the organization was sub-divided into several divisions respectively for cereal crops, horticultural crops, and special crops, and their evaluation data was augmented. In addition, chemical composition profiles such as oil and fatty acid content of sesame seeds, protein content, amylose and amylopectin content, andstarch characteristics of rice landraces, and DNA polymorphism analysis of rice and barley were initiated [8]. In soybean (*Glycine max*), the morphological characteristics of 650 landraces were investigated, and about 40 of them were selected as a core collection, and a molecular genetic profile was promoted [9]. Among the recent data updates

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of the passport information, significant achievements include the selection of strains resistant to the blight of red pepper (*Capsicum* spp), analysis of capsaicin content, cultivation of anthracnose-resistant intermediate seedlings, evaluation of Chinese cabbage (*Brassica rapa* ssp. *penkinensis*) landraces, cultivation of superior groups, and cultivation of intermediate seedlings resistant to wart disease of cabbage [10,11]. The germplasm with passport data evaluated until the year 2,000 accounted for 79% of the total germplasm, and rice, barley, miscellaneous grains and legumes were evaluated at 88-68%, while horticultural crop**s** were less than 54%. With further work carried out on expanding the information available in the evaluation data for germplasm conserved at the RDA-Genebank, substantial consideration has been given to using high-throughput techniques to study the germplasm. One such aspect is using digital phenotyping for large scale analysis and screening of germplasm.

## 5. Digital Phenotyping of RDA-Genebank

In antiquity, the significance of seed traits were widely acknowledged and seeds were selected based on their phenotypic characteristics to improve crop yield and quality, making it one of the oldest phenotyping techniques [12]. Phenotypic characteristics, such as seed size and shape, were considered important in agriculture as they had a direct influence on consumer preference and market value. Consequently, traditional breeders aimed for high yield, specific grain size, and shape, with long grains being preferred for rice , and large, spherical grains being favored for wheat due to their suitability for milling [13,14]. With the exponential growth of genomic information on plants, advanced phenotyping techniques are now required to complement the genotype data obtained from next-generation sequencing technology [15]. Phenomics technology also has made significant progress in the past 10 years in various aspects. However, the process of building phenotypic databases is time-consuming, resulting in a phenotypic bottleneck [16]. By constructing two-dimensional (2D) images using cameras and scanners as well as three-dimensional (3D) images using techniques such as magnetic resonance imaging (MRI) and computed tomography (CT), it has now become possible to analyze crop phenotypes in a detailed manner [17–21]. However, 3D imaging can be expensive and is not always practical, particularly for large numbers of genetic materials conserved in genebanks. By contrast, 2D imaging is a rapid and cost-effective method that can be used for a large number of germplasms. In this regard, the RDA-Genebank has adopted the use of ImageJ Software, utilizing 2D images constructed with RGB cameras to phenotype seeds (Figure 3). This method has been proven successful, with a high correlation coefficient ($r^2$) between image-based and actual height and width measurements of soybean seeds ($r^2$= 0.9735, $r^2$= 0.9839, respectively) [22]. Therefore, it can be applied to a large number of plant genetic resources conserved in the international genebank. In 2022, eight seed phenotype characteristics were measured using digital analysis techniques in 598 soybeans (*Glycine max*), Diversity analysis was performed using origin (Korea, China and USA) and status (landrace, cultivar and wild) [22] . Currently, 2D methods are employed to compare seed traits of Northeast Asian landraces, including Repubic of Korea, China, North Korea, and Japan, which are recognized as the primary origin of soybeans. Furthermore, chemical compositons profileing, such as antioxidant activity, is incorporated to validate the correlation between seed traits [23]. In genebanks, it is imperative to not only include the agronomic traits sought by consumers but also anticipate future research directions and continuously incorporate characteristic investigation items. Roots, which are fundamental plant organs responsible for absorbing water and nutrients, are crucial for supporting soil, crop productivity, and environmental stress acclimation, such as drought, and, as such, are indispensable plant organs for breeders. Nonetheless, roots are more challenging to study when compared to other parts of plants. In 2021, we endeavored to measure soybean(*Glycine max*) and adzyki bean (*Vigina angularis*) germplasm's root phenotype for the first time across international genebanks. Specifically, we cultivated 380 soybean landraces and 61 wild adzuki bean in polyvinyl chloride columns (PVC) pipes until the V2 stage, when the second true leaf emerged, and we constructed images with a scanner and analyzed root phenotypes with commercially available WinRIZO software [24–26]. We confirmed that eight root morphological traits (RMT) were distinctive for classification, and the accessions were divided into three clusters. Furthermore, it was feasible to provide breeders with information by selecting excellent germplasm in comparison to *Glycine max* cv. Enrei Japanese varieties, which are known for their excellent nodulation characteristics. Additionally, these digital phenotyping techniques can be applied to various other crops conserved at the RDA-Genebank. For instance, in wheat, RGB images obtained from a digital camera can be utilized to measure agronomic traits such as tiller number per plant , canopy, including leaf number and length, phenotypic characteristics of wheat spike, and tomato internode length. In the future, RGB images captured using unmanned aerial vehicles can also be used to measure plant heights in crops such as rice, wheat, barley, and corn [27–30]. Hyperspectral sensors have been utilized to measure photosynthesis and respiration, which are directly related to crop growth, and are currently being studied in tobacco, corn , and wheat [31–33]. In addition, biomass yield research has commenced by utilizing shoot color in wheat for biomass yield estimation and thermal imaging cameras for wheat tiller number [34].

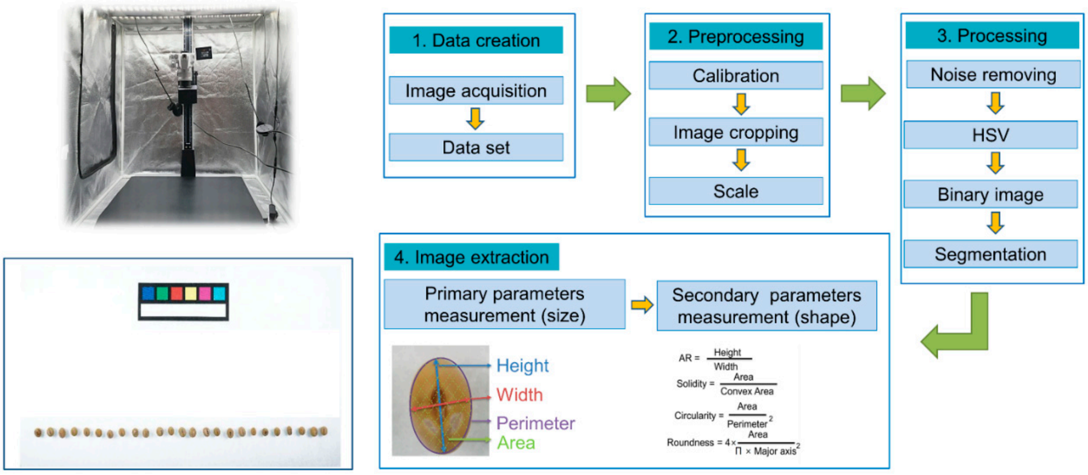

**Figure 3.** Flow chart of seed phenotyping using 2D image created by digital camera currently used RDA-Genebank [22].

The augmentation of phenotype data with existing characterization information, as well as the integration of genomic, phenomic, and other molecular data, can significantly aid researchers in selecting optimal accessions for breeding and elucidating various evolutionary characteristics [35]. In the case of rice, the combination of phenotyping data with genome-wide association studies has proven useful in understanding the genetic variation occurring in the crop [36,37]. At the RDA-Genebank, we conducted a genome-wide association study (GWAS) using root phenotype data and 180K single-nucleotide polymorphisms (SNPs), leading to the identification of 112 SNP loci related to seven root traits and the selection of 55 putative candidate genes (Figure 4) [38]. Phenomics is a vital component of the recently proposed concept of 'PANOMICS,' which seeks to integrate multiple '-omics' data sets for faster analysis and improved germplasm quality [39]. Therefore, in the near future, the RDA-Genebank plans to provide germplasm information in a combined format for end-users to facilitate the selection of appropriate germplasms.

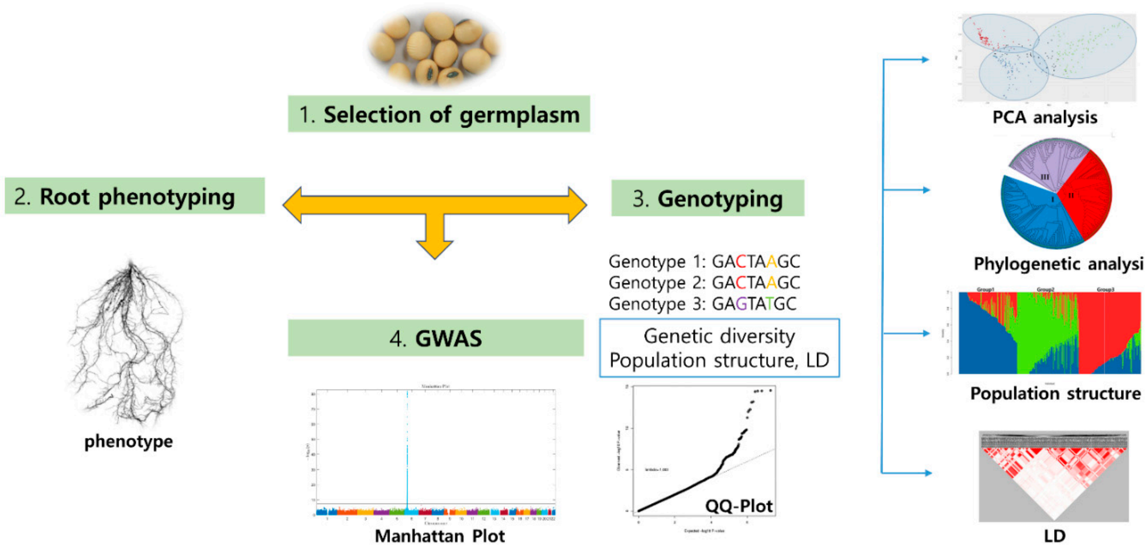

**Figure 4.** Flow chart of currently proposed germplasm research conducted by RDA-Genebank [38].

## 6. Conclusions

Given the accelerating loss in biodiversity worldwide due to changing climatic conditions and anthropogenic activities, the importance of agricultural genetic resources is becoming more prominent. At plant genebanks throughout the world, continuous efforts are being taken towards improving the quality and quantity of germplasm collection along with ease of access. The germplasm we conserve at the National Agrobiodiversity Center are continuously investigated for agricultural traits and building an updated database. Recently, we also concentrate on methods to investigate agricultural traits by applying digital techniques. We believe, these will lead us on our way in developing into one of the leading plant genetic resouece centers of the world in the future.

**Author Contributions:** prepared a draft of the manuscript, project administration, funding acquisition and supervison S.-H.K.; review and editing, P.S, H.-S.L., Y-W.N. B.-S.H.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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