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Review Paper

Intellectual Property Rights (IPRs) for Plant Breeders: A Review on Theoretical Framework and Employment of DNA-based Varietal Authentication for Claiming IPR

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Abstract: Plant patents (PPs) and Plant Breeders' Rights (PBR) are two forms of Intellectual Property Rights (IPRs) granted to improved novel crop varieties. The government of the state of authority issues PPs and PBR after confirming the uniqueness of varietal identity. The uniqueness relies on distinctiveness, uniformity, and stability of the new

variety. Morphological, physiological and biochemical descriptors are less capable in varietal discrimination to obtain IPR in the presence of large number of closely related varieties as the reference collections, but advanced molecular tools such as DNA fingerprinting and sequencing have high potentials to detect the uniqueness. DNA fingerprinting and sequencing have identified varietal identities of many crops such as rice, apple, wheat, and soybean revealing the potential of the successful use of molecular descriptors in granting patents or PBR. The novelty verification is the first step in the process of allowing patents or PBRs. The patent or plant variety protection office requires an application from the breeder that includes all the details of the plant variety fulfilling all statutory requirements to grant varietal ownership *via* a patent certificate or a plant variety protection certificate. Currently, Sri Lanka has no developed system of IPRs to allow PBR or patents for improved crop varieties. The efforts made by breeders in developing novel varieties can be justified and appreciated by granting plant varietal ownerships. For this purpose, molecular descriptors must be used instead of inefficient morphological, physiological and biochemical characters to avoid ambiguities and to clearly define the inventor of a particular variety.

Keywords: Crop variety ownership, DNA Fingerprinting of rice, Plant Breeders, Rights, PBR, Rice Varietal identity.



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Introduction

Development of the improved crop varieties is crucial to feed the increasing global population. Plant breeders play a vital role in producing novel

varieties as the production of a new variety through breeding requires hard work (Jennings et al. 1979). Breeding is a long-term highly expensive activity

and most of the experiments end up in failures rather than successes. The effort taken for the successful development of an improved variety must be honoured by offering a legal right to the breeders for the ownership of the variety that they developed. Plant Patenting (PP) and Plant Breeders' Right (PBR) are the two forms of Intellectual Property Rights (IPRs) granted to plant breeders. PP protects novel, distinct, asexually reproduced plants allowing the owner the freedom to prevent others from asexually reproducing or trading the newly bred plant (Bouchoux 2012; Williams 1984). PBRs protect sexually reproduced or tuber propagated crop varieties by granting Plant Variety Protection Certificate (PVPC) (Barton 1982).

Before granting either PP or PBR, the improved variety must be confirmed as being unique from existing crop varieties in morphological, biochemical or molecular attributes. The currently used criteria are the morphological and biochemical attributes to test distinctiveness, uniformity, and stability of the developed variety. Insufficiency of the phenotypic characteristics for varietal discrimination prevents the opportunity of obtaining IPR, thereby making it unable for breeders to benefit from their invention. However, compared to morphological descriptors, the

Intellectual Property Rights

The IPRs are rights awarded for specific products of intellectual attempt and ingenuity by a state of authority (Bhat 2006). IPRs cover designs, innovative creations, literary and artistic work. Four main forms of Intellectual Property (IP) are copyrights, trademarks, trade secrets and patents. Literary, dramatic, musical and artistic works are protected under copyrights which are granted to authors of original work having authorships. Trademark consists of a term, symbol or a device which indicates the ownership, quality and source of the product and trade secrets cover financial projections, marketing plans, business information and business conducting methods. The state grants a patent to a novel invention, which gives the owner a right to prevent others from manufacturing, marketing, and importing the product (Bouchoux

molecular techniques such as DNA fingerprinting and sequencing together with marker technology can provide higher discrimination power in differentiating very closely related varieties that show similarities in morphological traits (Paurabed *et al.* 2015).

IPRs are granted for improved varieties to claim the ownership in developed countries such as USA and UK by establishing various acts and agreements. Most developing countries such as Sri Lanka do not have a system of IPR to allow plant varietal ownership, which is required to inspire breeders and encourage investment in future breeding programmes. Lack of varietal protection indirectly will enable competitors in the private sector to make use and trade the new variety locally and even to introduce it to the international market without providing any share of the profit to the owners/inventors of the variety (Bouchoux 2012). Therefore, in the present review, we aim to provide a comprehensive knowledge about the process of acquiring PP or PBR for a newly produced variety to facilitate the introduction of a procedure to grant IPR for plant varieties in Sri Lanka. We also emphasize the roles that DNA fingerprinting and DNA sequencing can play in varietal discrimination for breeders to accurately claim the ownership.

2012). Other than that, plant breeders can obtain PBRs, which is similar to a patent in that it protects and authenticate novel plant varieties (Weising *et al.* 2005). Patents provide the strongest protection to genetically modified plants whereas PBR assure the greatest approach to protect the individual plant varieties. The origin of patents and other types of IPR are not very well known. But in England, the history of patents can be traced back to 15th century from which era, the patenting process has undergone continuous evolution with the rising technological development (Mayer 2003). Global communication has also become a significant challenge in protecting IP as they can be misappropriated (Bouchoux 2012) however, in contrast, globalization has also lead to synchronize the worldwide IPRs systems.

Patenting and patent types:

The patent is a privilege and a treaty that is built between the originator and the state with respect to an invention which gives the right to exclude others from enjoying the invention without the consent of the patentee. Patents can claim for new useful discoveries, creative designs, products, processes, and apparatus (Bouchoux 2012; Crespi 1982) however scientists do not patent entities such as scientific principles. There are several types of patents granted including utility patent, design patent, PP, reissue patent, defensive publication, and stationary invention registration.

The protection through patenting is valid for 20 years, and after that, the product falls into the popular domain to utilize without any permission from inventors (Bouchoux 2012). The primary purpose of a patenting system is to stimulate investments in invention and technical progress, increase the productivity growth, design of new products and their marketing by providing monopolistic power to the originator and encouraging early disclosure of the product (Griliches 1990) while enabling the inventor to return the cost for developing his product. The authorities assess the patentability of an invention for novelty, inventiveness, utility or industrial applicability and no patents allowed to the designs with minor alterations of existing products (Bouchoux 2012; Ginarte and Park 1997).

A dedicated government department/office identified as 'Intellectual Property Office' or 'Patent Office' controls the patent granting of a country. The patent office has rules set by statute and regulations established for practical operation. Each country has its national patent law and practice, and the legal effect is territorial over which the state has authority (Crespi 1982). The prosecution of a patent is costly, complicated and time-consuming and for United State Patent and Trademark Office (USPTO), it takes about three years to issue a patent (Bouchoux 2012). The international treaties for patenting are in place. Such agreements include Paris Convention of 1883 and its revisions which provides national treatments for foreigners in offering patent rights, Patent Corporation Treaty (PCT) of 1970 involving in delivering a patent application which is

acceptable in any patent office of a member country and International Union for Protection of New Varieties of Plants (UPOV) in 1961 protecting newly developed plant varieties (Ginarte and Park 1997).

Utility patent:

The utility patent, also known as utility model, petty patent or innovation patent is the most common patent type covering a wide variety of inventions and discoveries. These patents are easy to obtain and maintain (Beneito 2006), and the protection endures for 17-20 years. The utility patents protect novel and fruitful processes, machines, the production or the composition of a substance (Bouchoux 2012; Spinello 2007). These patents provide the most effective form of protection for transgenic plants and their products, and methods producing transgenic plants. (Kjeldgaard and Marsh 1994). The genuine process of invention develops a modern route or a design of new operating conditions for a known process (Crespi 1982). However, scientists often claim that the further use of already existing process (Bouchoux 2012) in which the novelty is only within the application (Crespi 1982).

The product patents are the most effective form of protection, which claims for substances, and 'product per se' grants the protection for new substance whereas 'product by process' claim for a product directed through a defined process (Crespi 1982). The prior use of protected invention commercially may requires approval from government agencies such as Department of Agriculture or Food and Drug Administration (Kjeldgaard and Marsh 1994).

Design patent:

Design patents protect innovative, creative, object-oriented ornamental designs for an article of manufacture (Bouchoux 2012; Spinello 2007) where object-orientation directs the plan to a concrete object, not a theoretical draft. The design is a visual creation completed in respect of shape, stain, and the pattern of an artifact having a short life cycle due to the dependence on ornamental appearance and market demand (Chen and Chen 2004). Therefore, the protection persists only for 14 years from the date of grant, which is a relatively

short duration (Bouchoux 2012). Depending on aesthetic appearance, design patents protect ornamental features rather than functionally covering many aspects of the industrial constructs (Lee and Sunder 2013) including furniture, jewelry (Bouchoux, 2012), tools and automobile designs (Kluth and Lundberg 1988). Compared with other IPRs, design patents are faster and easy to obtain providing robust and expansive protection. However, there are limitations such as expensive, an effort involving process, relatively high substantive standards for securing patent (Lee and Sunder 2013) and subjectivity of identification due to different forms and shapes (Chen and Kuo 2013). Through searching of current design patent data published by IP organizations in developed countries, falling others' patent scope can be prevented, and the inventors can enhance the originality of the design by understanding the current level in the market (Chen and Chen 2004).

Plant patent (PP):

The PP protects novel, distinct, asexually reproduced plant varieties for 20 years conferring the owner the right to exclude others from asexually reproducing or vending the plant (Bouchoux 2012; Williams 1984). Until 1930, plants did not get patented as they are products of nature and thought not to be flexible to the written description requirements of the patent law (Blair 1999). Since asexually reproduced plants are genetically identical to their parents, after verifying their distinctiveness and uniformity achieved through breeding, asexually reproduced plants have protected legally through the US Plant Patent Act of 1930 (Barton 1982). Plants propagate through roots, stolon, rhizomes, stem or leaf cuttings or tissue culture can be patented (Blair 1999) excluding plants propagated through tubers, discovered in the wild and reproduced utilizing seeds (Bouchoux 2012).

A novel plant should be discrete from existing varieties with distinctive features in morphological or biochemical characters (Kjeldgaard and Marsh 1994) and a vegetatively propagated clone bearing a genetic mutation with identical heredity and have characteristics for PP (Blair 1999). Thus, PPs protect asexually reproduced plants including

ornamental and fruit-bearing trees (Sechley and Schroeder 2002).

Reissue patent:

Reissue patents confer the already published patents to amend the claims of the original patents (Bessen 2008). A patent is reissued to rectify the deficits in the first utility, design or PP. Defective specification, illustration or variations in the claims are some of them due to which the patent is determined to be partly or entirely inoperative. However, the patentee can apply to reissue the patent only if the deficiency has occurred inadvertently. The application should be delivered within two years from the date of bestowing of the original patent which persists for the unexpired duration of the initial patent (Levine et al. 1996). Through reissue patent, the applicant can claim for whole invention or drop questionable and invalid claims (Woodward 1948). With the surrender of original patent, the inventor should establish proper justification for the assessment (Levine et al. 1996). Therefore, a sort of resistance leads to a definitive end ensuring no further expansion of the patent while leaving a zone of uncertainty for the competitors for two years (Lemley and Moore 2004).

Defensive publication:

Defensive publication is a strategy in which new inventions originated by scientific research is being made public. Thus, the inventor exploits new knowledge securing the freedom of right to practice the origination in the first place. As in defensive publication, the invention is unconfined in the form of written or electronic publication as annual reports, journals and websites, a patent cannot be awarded due to lack of novelty (Adams and Apollonio 2002). Therefore, the disclosure of new scientific findings establishes prior modern art affecting the patentability of related innovations (Bar 2006), making the competitors' invention visible (Barret 2002).

In a defensive publication, originators disclose the information with the help of a third party. The inventors assure the easy access for the target audience with an unambiguous publication date by publishing a comprehensive description of the entire innovation quickly. Compared with patents,

a defensive publication is less costly, and in agricultural research, it helps to communicate the results to others as well as to forestall the eventual patent award disseminating scientific knowledge successfully (Adams and Apollonio 2002).

Statutory invention registration (SIR):

The SIR gives an approach of invention registration that has the protective aspect of a patent rather than the enforcement aspect (Bouchoux 2012) where it provides the inventor an inexpensive way of creating prior art (Oppenheimer 2015). The SIR was first founded in 1984 replacing the defensive publication and mainly operated by US federal agencies as a mode of disclosing materials to the public domain (Adam and Apollonio 2002). The SIR grants when there is no interest in enforcement of an invention although the expense of obtaining a

patent can be paid, or to avoid another inventor getting a patent for the same design. The inventors do not need novelty and non-obviousness securing an SIR and an application including the method of formation and utilization with drawings to understand the invention is the only substantive requirement (Oppenheimer 2015). Upon publication of SIR, the inventor will surrender all the rights on design, and he or she will not be able to preclude others from creating, marketing, and utilizing the invention (Bouchoux 2012). The SIR has an added benefit of considering the date of submission of the application to the patent office as the date of publication rather than the published date. Though SIR benefits the public, the inventors seldom used it compared to other patent types. Thus, SIR got repealed in 2011 (Oppenheimer 2015).

The Patenting Process

The prosecution is the process of preparing, filling and shepherding a patent application towards its issuance. The inventor or a patent-attorney can fill the application. In the USA, after completing the patent application with USPTO, the application will be examined by more than 6000 patent examiners who are specified in the technology related to the invention to verify that the design complies with the statutory requirements for patents. The 'Office Action' is the document sent to the applicant after examining the application including the objections, and if the response given to the Office Action is acceptable, the applicant will receive an allowance notice specifying the issue fee charged by USPTO for patent granting. In general, the prosecution takes about three years. Then the authorities publish necessary information about the patent, and they issue the patent certificate (Bouchoux 2012).

As novelty and non-obviousness are essential requirements for patent issuance, the novelty search or a prior art search is necessary to verify whether the subject matter is already patented, or it is open to public domain due to the expiration of the granted patent. In both cases, application for the same subject matter is not useful due to lack of novelty and obviousness. Although it is not a critical requirement to carry out a patent search

prior to filling the application, feasibility of obtaining a patent can be determined which is beneficial for the applicant as the process of getting a patent is expensive. Also, prior art search will provide ideas for drafting the application itself. Furthermore, freedom to operate search can be conducted to confirm whether the invention infringes any existing patent if the invention is used commercially in the future (Bouchoux 2012).

Preparation of plant patent application:

Among different types of patent applications such as provisional applications, utility applications, design applications, continuing applications, continuation applications and patent cooperation treaty applications; plant patent application seeks protection for new, distinctive and vegetatively reproduced plants. The paperwork for the patent must be exclusively in English, and it has elements such as specification, drawings, models, specimens and oath of the inventor. The complete description of the invention is given in the section of the specification which is further divided into sections including title, summary, detailed description and claim. The title is short including the specific name of the invention. The summary provides a brief statement of the nature of the invention and its advantages. A detailed description is included explaining the process of making, usage with clear,

concise terms and assertion to disclose the details of the origination. An oath or declaration must be signed stating that the applicant believes himself or herself as the foremost originator of the invention.

Plant Breeders' Rights (PBR)

In plant varietal improvement, when introducing a new variety, a breeder can apply for PBR (Weising *et al.* 2005). It protects plant varieties propagated through seeds and tubers (Barton 1982) and Essentially Derived Varieties (EDVs); the ones primarily derived from an original variety or from a variety itself derived from a first variety conserving the expression of important traits resulting from the genetic architecture of the parental variety (Ardley and Hoptroff 1996). Many countries have enacted Acts and International Agreements to establish PBR within their territory and US Plant Variety Protection Act (PVPA) of 1970, provides breeders with a certificate granting rights for plants defined concerning uniformity, stability, and distinctiveness (Barton 1982). UPOV established in 1961 by European Community confers PBR similar to a patent (Ginarte and Park 1997) whereas the United Kingdom introduced PBRs through Plant Varieties and Seeds Act of 1964. The protection of plant genotypes and Farmers' Rights Act (2001) and the Biological Diversity Act (2002) are some of the regulations which strengthen the rights of farmers as well as the breeders (Bhat 2006). All these acts and treaties offer breeders excluding others from producing, breeding, trading, importing or exporting of the propagating material of the protected cultivar (Ardley and Hoptroff, 1996) to safeguard their privileges.

Plant variety protection certificate (PVPC):

A patent like a certificate called PVPC is granted to a novel, sexually reproducing and tuber propagating plants other than fungi and bacteria under US PVPA, 1970 (Alston and Venner, 2002). Plant Variety Protection Office of US Department of Agriculture administrate the awarding of PVPCs to new crop cultivars (Rongwen *et al.* 1995). PVPA protects the cultivar for 25 years from the date of issuance for vines and trees and 20 years for other crops (Carpenter 2005). To acquire the certificate, a breeder must apply with a complete report of the

Furthermore, the applicant must also provide an address identifying his or her country of citizenship (Bouchoux 2012).

plant variety comprising the breeding history and seed deposit for viability testing *via* propagation (Blair 1999). Multiple PVPCs are not issued for the same plant variety, therefore, upon submission of two or more applications on the same effective filing date where the plant varieties are difficult to differentiate clearly, PVPC would be granted to the applicant who completes all the requirements of PVPA (Carpenter 2005). As required for utility patents, which also protect entirely differentiated plant seeds as well as multiple parts of the plant that reproduce through seeds, PVPCs does not require usefulness or non-obviousness for protection, therefore, achieve relatively a weaker protection (Alston and Venner 2002). Although in the US, PPs are granted for vegetatively propagating plants, in other countries PVPCs are used for this purpose as well (Wright and Pardey 2006).

The process of obtaining PVPC in the USA needs to fulfill three requirements; completion of all the application forms, introduction of a variety name which does not conflict with already existing plant varieties and deposition of seeds or tissue cultures, 3,000 viable untreated seeds of the variety or samples of live tissue culture of the variety including ten firmly rooted (four to six weeks old) *in vitro* plants. The application should include all the information about plant color, qualitative and quantitative traits as well as the details on the disease, insect and environmental resistance of the novel variety. After examination of the novelty, uniformity, and stability, PVPC is granted providing exclusive right and protection for the variety for 20 years. Table 1 presents details of some of the varieties given with PVPCs (USDA 2018).

Organizations protecting plant varieties:

National and international organizations have established agreements for safeguarding plant varieties to stimulate breeders to invest in developing new cultivars to meet the increasing

demand and make progress in agriculture. World Trade Organization (WTO) which originated in 1995 administrates various agreements related to IPR (Bouchoux 2012) and countries that are signatories to WTO agree on the conditions of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPs), which comprises minimum standards for IP protection and effective enforcement procedures. Even though animals, humans, plants and essentially biological processes for the production omitted

from patentability, adherents must offer protection for plant varieties either by patent or efficient *sui generis* system (Bowman 2007; Tripp et al. 2007). Most countries adopt PBR based on UPOV where their one of the primary functions is to coordinate legal and ethical aspects of PBR providing guidelines for the test for the novelty of a cultivar, currently to have instructions for more than 250 different species (Cooke and Reeves 2003).

Table 1. Details of PVPCs issued for some novel varieties

Crop	Application number	Variety name	Patent holder/owner
Rice	201000006	CL111	Louisiana State University Agricultural Centre
	201000072	Hikarishinseiki	Motonori TOMITA
	201100296	Jazzman-2	Louisiana State University Agricultural Centre
	201300457	Colorado	Texas AgriLife Research; State of Texas Research Agency
Soybean	201400440	1046909	Monsanto Technology, Limited Liability Company.
	201400439	1046852	Monsanto Technology, Limited Liability Company.
Cotton	201100410	A1027054	Monsanto Technology, Limited Liability Company.
	201100409	A1027048	Monsanto Technology, Limited Liability Company.
Pepper.	201300372		Seminis Vegetable Seeds, Incorporation
Wheat	201100466	WB-Tucson	Monsanto Technology, Limited Liability Company.
Barley	201200011	ABI Voyager	Busch Agricultural Resources, Limited Liability Company.
Bean	201100450	Bellagio	Michigan State University
Peanut.	201200100	Georgia-11J	University of Georgia Research Foundation, Inc
Sorghum	201400199	Phonplmie	Pioneer Hi-Bred International, Incorporation
Squash	201200299	CAS1301076	Seminis Vegetable Seeds, Incorporation.

Source: USDA (2018)

UPOV and UPOV convention:

Six European nations formed UPOV in 1961 *via* a convention (UPOV convention) to provide a legal context for PBR legislation (Blair 1999). Under UPOV convention, Plant Variety Protection (PVP) is established after registration which requires distinctiveness, uniformity, and stability of the original variety (Jordens 2005). The convention came into effect in 1968 and amended in 1972, 1978 and 1991 (Wright and Pardey 2006), and all three treaties contain standard criteria, minimum scope, and duration for protection and the

minimum number of plant species and genera for which protection is granted. Member counties classified as developed, newly industrialized and economies in transition (Tripp et al. 2007) and later broadened into five classes in 1972 (Jordens 2005). As by 2005, among 55 states which were parties to UPOV, 29 countries are signatories of 1978 and 24 are signatories of 1991. Therefore, two most reason acts are briefly discussed.

According to the 1978 act, states are required to protect five genera or species at time of accession

increasing it up to 24 within eight years later. Compared to that, 1991 Act requires number of genera or species to be protected at the time of accession as 15 covering all the genera or species within 10 years. Protection was granted only for selected plant varieties for 15-20 years in 1978 whereas in 1991, it was broadened to grant protection for all plant genera or species for 20-25 years allowing breeders to share the rights for EDVs for which protected variety has been used. Furthermore, under 1978 Act, plant variety could be protected either by PBR or plant patent whereas under 1991 Act, both types of protections are allowed for the same variety (Tripp et al. 2007).

Distinctiveness uniformity and stability testing:

Commercial introduction of a new variety requires registration, which is governed by the criteria Distinctiveness, Uniformity, and Stability (DUS). Establishing DUS test is a key to identify novel variety from a reference collection to grant PVP (Paurabed et al. 2015). A distinct type differs from varieties in the public domain in one or more desired physiological, morphological or other characteristics provided by the guidelines of UPOV (Kjeldgaard and Marsh 1994; Williams 1984). The instructions are mostly field-based observations as plant height, the color of leaf and flower petals consisting of the mixture of continuously and discontinuously expressed descriptors (Cooke and Reeves 2003).

Uniformity of a variety is achieved if the characteristics are predictable and commercially acceptable and a stable variety can be reliably reproduced through seeds exhibiting main and distinct characters unchanged (Kjeldgaard and Marsh 1994; Williams 1984). Even though DUS test can determine the uniqueness of a variety, restrictions arise due to morphological descriptors used in setting the identity, which is influenced by the environment, increasing number of existing varieties, time-consuming and expensive process requiring large land areas to cultivate plants

Plant Variety Licensing

The improved plant varieties developed by breeders can be commercialized through plant variety licensing. Access to novel variety *via* proper

(Tommasini et al. 2003). Thus, it is challenging to define sufficient distinctness between the new variety and the reference collection (Weising et al. 2005). However, in DUS testing, molecular markers can be used in place of phenotypic characters to facilitate efficient, profitable procedure for evaluating novelty (Paurabed et al. 2015).

Plant breeders' ownership:

Plant breeding is crucial in improving agricultural productivity (Ardley and Hopcroft 1996) and development of a novel variety is a work of 10-20 years with highly expense where the cost of breeding increase continuously for producing a new variety. Many of the experiments end up with more failures than successes. Before the new variety reaches to the farmer's field, thousands of experimental lines are evaluated and discarded, and even after introducing the novel variety, the breeder has to confront high competition to sell the reproductive material of his variety in local and export market (Hanson et al. 2014). Therefore, this hard work requires dedication, commitment and the patience of plant breeders throughout the work (Jennings et al. 1979). Appropriate credit should be given for their effort by granting ownerships through PBR for their novel varieties to provide encouragement for developing superior varieties (Carew and Devadoss 2003). The beneficial traits of these varieties may promote progress in agriculture (Blair 1999). A breeder granted with PBR has the monopoly of producing the variety of commercial marketing, exporting the propagative materials and offering for trade (Crespi 1982). However, compared with plant and utility patents, ownership granted for breeders through PBR differ in two aspects called 'breeders exemption' and 'farmers' exemption.' Breeders exemption allow the use of protected variety for research in breeding and the farmers' exemption enable farmers to keep seeds to save in subsequent growing and sell a proportion to other farmers whose primary occupation is growing crops for feed (Pardey et al. 2013).

handling of intellectual property can be obtained through plant variety license, which is an agreement signed between the variety owner and a

legally entitled person with a desire to trade the variety. Distribution licenses and production licenses are two types of plant variety licenses where the former provides privileges to advertise and vend the licensed object and the latter allows manufacturing and proliferation of licensed material in addition to rights granted by distribution licenses (Krattiger et al. 2007). The permit issued may be either exclusive, providing rights to utilize the plant variety only for one party or non-exclusive providing the rights for more than one party (Bouchoux 2012). Due to the economic benefit of a good variety, offering an exclusive license grants greater profit with best possible market exposure compared with a non-exclusive license which has a small market perspective. As trading a new variety before strengthening it with PBR may exclude the eligibility for PVP, distribution rights of license holder should be restricted to unprotected variety. A license may be offered to one variety, specific or entire crops varieties of a licensor and the issued permit may be limited regarding scope, duration, terms and territory (Krattiger et al. 2007).

In-licensing:

In-licensing is one of the two driving forces of licensing through which the plant varieties gain the ability to meet the consumer requirements as well as to enhance the variety portfolio of a business firm. In-licensing strategies are operated for plant genotypes which are under use in breeding

programs with potential applicability in trading. It usually includes a business firm obtaining a license from the owner of the variety for a novel, nutritionally and agriculturally improved genotype in the fulfilment of demand from farmers, processing industries, and consumers. In-licensing opens the path of achieving innovative knowledge such as hybrid plant varieties where business firms can use the novel technology without access to use licensor's breeding platforms, and this strategy can be applied to business firms both involved in their breeding procedures and exclusively work on in-licensed varieties (Krattiger et al. 2007).

Out-licensing:

Out-licensing is a strategy used by minor and moderate scale business firms to offer licenses of their novel plant varieties. The primary objective of granting out-licenses is efficient exploitation of result of the breeding platform allowing it to achieve its complete utilization potential. As small and medium level firms may lack capital and other resources to establish large-scale organizations within their own country and other countries to spread their invention and advertise it successfully, out-licensing provides a fruitful means of distribution of their novel plant varieties. By permitting other organizations to manufacture and trade the plant variety, they may obtain the total profit or make the best use of their asset placed for the improvement of the new variety (Krattiger et al. 2007).

DNA Fingerprinting for Patenting Plants and Animals

Development of new plant or animal either by conventional breeding or with the help of recombinant DNA technology makes breeders eligible to be granted with patent rights. Morphological, developmental and physiological traits to identify are subjective and quantitative (Wang et al. 2010). Molecular markers play a valuable role in differentiating varieties (Congiu et al. 2000) due to the availability of a considerable number of potential polymorphic sequences, which are directly applicable for IPR submissions (Thomas et al. 1993). In 1982, the US Supreme Court declared that livestock, non-naturally occurring organisms including new transgenic

animals are also patentable (Hettinger 1994), keeping the concept that products of nature are eligible for patenting if they are claimed in the form that they do not occur in nature (Elliott 2002). As novelty, non-obviousness, and utility are required to issue animal patents, the animal should be described verbally, graphically, genetically and other methods (Czarnetzky 1988). The search for prior art involves searching for DNA sequences or protein sequences against databases (Elliott 2002) where DNA fingerprinting is used to assess the relatedness among individuals (Alford and Caskey 1994).

Microsatellite markers:

Microsatellites or Simple Sequence Repeat (SSR) markers are 1-6 nucleotide long tandem repeat motifs (Parida et al. 2009) distributed uniformly in eukaryotic genomes including plants (Logerstrand et al. 1993). Microsatellites show high polymorphism due to differences in the number of tandemly repeating units (Morgante and Olivieri 1993; McCouch et al. 1997) which arise due to replication slippage or unequal crossing over in meiosis. The designing of primers for flanking regions allow locus-specific amplification (Parida et al. 2009). Compared with other molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD), the expected heterogeneity in SSR markers is high (Powell et al. 1996). Their high informative nature, simple Mendelian inheritance, ability to be analyzed using rapid simple, inexpensive PCR based assay (Brown et al. 1996) and physical linkage to expression genes representing a functional marker are advantageous attributes for the use of SSR markers. The only disadvantages are their high cost of cloning and the enrichment procedures mandatory for their generation (Worhmann and Weising 2011). Hyper-variability, co-dominant inheritance, high reproducibility, simplicity, reliability and high allelic diversity have made them the choice of marker for various applications especially DNA fingerprinting of plants and animals (Parida et al. 2009).

Microsatellite markers are used in high-density genome map construction in combination with other PCR-based molecular markers (McCouch et al. 1997).-McCouch et al. (2002) have developed and mapped 2240 microsatellite markers in a high-saturated rice genome map. In plants, the highest numbers of microsatellite markers have been detected in maize, tobacco, soybean, pea and *Arabidopsis thaliana* (Morgante and Olivieri 1993). Microsatellite markers are also being used in gene and Quantitative Trait Loci (QTL) mapping and Marker Assisted Selection (MAS) (Zhang et al.

2012). Because of the high abundance and broad distribution across the genome (McCouch et al. 1997), microsatellite markers are applied in genetic diversity analysis of rice (Jain et al. 2004), wheat (Huang et al. 2002) and many other crops. Plant varietal identification, paternity determination, establishing evolutionary genetic relationships and population studies (Parida et al. 2009; Morgante and Olivieri 1993) are the other applications of microsatellite markers. With many critical genetic attributes and wide-ranging practices, microsatellite markers are considered as valuable genetic markers in the field of molecular biology.

DNA fingerprinting for varietal identification:

DNA fingerprinting where DNA based methods generate distinctive patterns of DNA fragments in differentiating plant varieties (Smykal 2006) is advantageous, because phenotypic identification is expensive, labor-intensive and environmentally variable with limited variance associated with many traits. Progress in marker technology leading to the development of RAPD, RFLP, AFLP, and SSR has resulted in several benefits for identifying cultivars (Kwon et al. 2005). The marker class RAPD is very popular in identifying varieties where nothing or very little is known about the genome sequence (Smykal 2006) giving a rapid, straightforward approach to detect polymorphism (Yang and Quiros 1993). Varieties of barley (Fernandez et al. 2002), apple (Landry et al. 1994), olive (Besnard et al. 2001) have been identified using RAPD markers. RFLP markers detect a high degree of polymorphism and stability. The high polymorphic rate per run, low cost and high reproducibility of AFLP making it suitable for the identification of vegetatively propagating plants (Zhang et al. 2000). The SSR is the marker type used widely in variety identification including plants such as soybean (Rongwen et al. 1995), wheat (Perry 2004) and rice (Shi et al. 2005). Therefore, DNA fingerprinting together with marker technology significantly increases the speed of variety identification.

DNA Sequencing for Patenting Plant Varieties

Patenting life forms including plants, animals, and micro-organisms based on molecular tools increase the discrimination power as well as the accuracy in determination of uniqueness. Although DNA fingerprinting can successfully differentiate life forms, sequencing of amplified loci in the genome would further assist the process in living being. The idea of using DNA sequencing for patenting was first accomplished in 1990's in which patenting of human DNA sequences or genes was proposed. It was suggested to grant a patent for human genes *via* sequencing of random complementary DNA (cDNA) called as Expression Sequence Tags (EST) with known function as cDNA has synthetically derived sequences rather than products of nature. Since then, thousands of human DNA sequences have been patented worldwide confirming the ability to use DNA sequencing for patenting genes as well for other living beings (Gitter 2001). As identification of plant varieties are also possible with DNA sequencing followed by polymorphism analysis in DNA sequences, application of DNA sequencing is a promising technique for granting patents for plant varieties and animals which are the outcome of breeding programs.

Sanger/dideoxy method:

The Sanger dideoxy method, the chain termination method developed by Sir. Fred Sanger and his colleagues (Sanger et al. 1977) is the most widely used method for sequencing (Fakruddin and Chowdhury 2012) with continuous improvement and refinement since its advent in 1977 (Gharizadeh et al. 2006). The sequencing is based on enzymatic chain termination by 2',3'-dideoxyribonucleotide triphosphate (ddNTP) (Hattori and Sakaki 1986) which has an inhibitory activity on DNA polymerase I when it is incorporated in place of deoxyribonucleotide triphosphate (dNTP). The lack of 3' hydroxyl group in the dideoxy helps terminate the chain extension (Sanger et al. 1977). The 3' H prevents chain elongation as phospho-diester bond cannot be created with the next ribonucleoside. Either forward- or reverse-DNA primer gets hybridized to the 5'- end of the single-stranded DNA to be sequenced (template), which permits DNA

polymerase to attach and synthesize a new DNA chain along the template through complementary base pairing. During Sanger sequencing, four separate reaction mixtures, each containing one of the four types of ddNTP oligonucleotide primers reacting with the template strand in the presence of all dNTPs in which one type of dNTP is labeled with P32. The mixture is incubated with DNA polymerase I to synthesize nested set of DNA fragments having primer defined 5' ends and 3' ends (Prober et al. 1987) terminated with ddNTP differing in nucleotide monophosphate units (Metzker 2005). Synthesized fragments in each mixture are separated on the high-resolution polyacrylamide gel to produce the autoradiographic image to observe banding pattern and thereby infer the nucleotide sequence (Innis et al. 1988; Sanger et al. 1980).

For inserting template DNA, bacteriophage vectors (M13 series) are used, and improved phage vectors have been developed due to deletions occur from large DNA inserts. In Sanger sequencing, dideoxy derivatives and arabino-nucleosides have been used as terminating triphosphates. An appropriate ratio of dNTP to ddNTP should be used to obtain suitable banding patterns from which extensive sequence information can be obtained. With Sanger method, sequences of length of 15-200 bp (base pairs) can be read from priming site with reasonable accuracy (Sanger et al. 1982).

Automated Sanger-sequencing for identifying plant varieties:

Introduction of automation techniques for one or more time consuming, expensive, labour-intensive steps as sample preparation, detection and interpretation (Prober et al. 1987) is vital in sequencing to read large DNA sequences (300-1000bp) or to determine short DNA sequences in a reliable manner (Trainor 1990). In automated Sanger sequencing, either primer or chain terminator ddNTPs with fluorescent dyes label the DNA fragments. Capillary Array Electrophoresis (CAE) (Metzker 2005) which is excellent for sensitive DNA analysis facilitating high resolution (Kheterpal et al. 1996) then separates the fragments. Fluorophores in labeled fragments

which provide sufficient sensitivity for recognition of the small amounts of DNA (Smith et al. 1986) are excited by laser beam to detect the emission spectra of four different colours emitted by each fluorescent dye. Digitized signals from detectors are used to analyse baseline corrected peak intensities through which four reference values are shown as peaks and order of fluorescent fragments determine the nucleotide sequence (Prober et al. 1987; Metzker 2005).

The success in large-scale sequencing projects in diverse fields of research including medicine, forensics, epidemiology and evolution (Metzker 2005) is dependent on the improvements in speed and automation of the technology. Smith et al. (1986) has developed automated sequencing method using four different dyes namely fluorescein, 2-(4-nitro-2,1,3-benzoxadiazol-7-yl)aminoethyl]trimethylammonium (NBD), tetramethyl-rhodamine and texas red to label primers. However, labelling chain terminating ddNTPs is advantageous because four sets of fragments can be generated within a single reaction mixture replacing four labeled primers with a single unlabelled primer (Trainor 1990). Development of family of fluorescent dyes 9-(carboxyethyl)-3-hydroxy-6-oxo-6H-xanthenes (Prober et al. 1987), fluorescent detectable primers that exploit fluorescent energy transfer to optimize absorption and emission properties of the label (Ju et al. 1995) and detection of primers with four color confocal capillary array scanner (Kheterpal et al. 1996) are some lines of progress in automation.

Use of four different fluorescent dyes has several disadvantages such as variation in electrophoretic mobility, spectral overlaps between dyes, inefficient excitation a single laser source and significant loss in fluorescent signal intensities due to use of band pass filters for detection (Ansorge et al. 1987; Lewis et al. 2005). However, an effective method called Pulsed Multiline Excitation (PME) in which labelled fragments are excited with a sequence of peaks from four monochromatic laser sources have helped to overcome some of these disadvantages (Lewis et al. 2005). Improvements in fluorescent dyes, enzymology, recognition, capillary array electrophoresis and rapidity in

automated Sanger sequencing (Metzker 2005) have broadened its area of applicability.

Applicability of sequencing in plant variety identification:

Variation in DNA sequence is the basis for genetic diversity accounting for a significant fraction of observed differences in plant varieties including their growth, yield, stress tolerance and dietetic excellence including naturally occurring genetic variations consisting of small insertions and deletions as well as base substitutions (McNally et al. 2006). Simple Sequence Length Polymorphism (SSLP) of microsatellite regions is mostly used in cultivar identification due to their polymorphism for length (Shirasawa et al. 2006). However, cultivars with point mutations cannot be identified by SSLP which is only possible by studying genomic variations among plant varieties *via* DNA sequencing (McNally et al. 2006). Sequencing of genomic DNA in small and moderate-sized samples of plants have revealed a significant fraction of variation within species (McNally et al. 2009). Identification of melon cultivars by EST-based sequence variation analysis (Deleu et al. 2009) is an example for successful applications of DNA sequencing in varietal identification.

Applicability of sequencing in rice variety identification:

Domesticated rice, *Oryza sativa*, with its well informative genome, is useful in genotype discrimination with the innovative use of genetic diversity (McNally et al. 2009). Due to well distributed single nucleotide variations across the rice genome (Shirasawa et al. 2006); DNA sequencing is a valuable technique to differentiate firmly related rice varieties. According to Nasu et al. (2002), single nucleotide variations are present in one in every 89 nucleotides in rice and chromosome number one, four, five and seven contain regions rich in individual nucleotide variations which can be used in cultivar identification. Shirasawa et al. (2006) were able to discriminate 21 rice genotypes *via* DNA sequencing followed by analysis of nucleotide deletion occurring due to frame-shift and missense mutation and sequence variation analysis in untranslated regions. Furthermore, a mutant rice variety of *Fujiminori* named as *Reimei*, was

identified with a trans-version mutation in gibberellin synthase gene (Sasaki et al. 2002). A mutant of *Koshihikari* rice variety designated as Milky Queen carrying a transition mutation in granule-bound starch synthase I (Sato et al. 2002) produced by crossbreeding can be only differentiated by DNA sequencing followed by nucleotide variation analysis. Therefore, rice varietal identification can be successfully achieved via DNA sequencing.

Single Nucleotide Polymorphism (SNP) as a tool of variety identification:

In a broad sense, SNP is defined as any single base substitution, insertion or deletion in the genome of an individual (Primmer et al. 2002). The SNPs are distributed in coding or non-coding regions of nucleic and plastid DNA and represent the most frequent type of marker used for the detection of

Conclusion

Among different forms of IPRs including copyrights, trademark, trade secrets, patents and PBR, the government patenting bodies grant novel varieties with plant patent or PBR by determining the distinctiveness, utility, and stability test (DUST). The breeders generally declare the uniqueness based on morphological, physiological

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the smallest unit of genetic variation within species (Vignal et al. 2002). SNPs are mostly bi-allelic, and their genotyping involves very high-throughput technology (Consolandi et al. 2007). Genome-wide polymorphism detection has become easy due to their abundance, stability in inheritance, reproducibility, and cost-effectiveness (Rafalski 2002). The SNPs are advantageous in cultivar identification as two or more related individuals are separated depending on the nucleotides occurring in a particular position of the genome (McCouch et al. 2010) enabling the clear-cut differentiation of very closely related varieties. In *Arabidopsis thaliana*, 4000 SNPs identified two varieties (Drenkard et al. 2000) and in olives, 17 SNPs identified 49 cultivars. Furthermore, Cabezas et al. (2011) identified 48 SNP markers able to recognize grape wine cultivars precisely.

and biochemical descriptors and run into ambiguities and failures to secure IPR due to the presence of closely look-alike varieties. Thus, modern plant patenting or PBR processes must use molecular descriptors such as DNA fingerprinting and sequencing to define the uniqueness of varieties/cultivars in a clear-cut manner.

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