植物品种特异性、一致性和稳定性测试15周年论文集

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中国农业出版社

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图书在版编目（CIP）数据

植物品种特异性、一致性和稳定性测试15周年论文集 / 农业部科技发展中心，农业部植物新品种测试中心编．—北京：中国农业出版社，2016.9

ISBN 978-7-109-21998-4

Ⅰ.①植…　Ⅱ.①农…②农…　Ⅲ.①植物—品种—中国—文集　Ⅳ.①Q94—53

中国版本图书馆 CIP 数据核字（2016）第 189446 号

中国农业出版社出版
（北京市朝阳区麦子店街 18 号楼）
（邮政编码 100125）
策划编辑　闫保荣
文字编辑　李　晓

北京印刷一厂印刷　　新华书店北京发行所发行
2016 年 9 月第 1 版　　2016 年 9 月北京第 1 次印刷

开本：889mm×1194mm　1/16　　印张：18.5
字数：700 千字
定价：60.00 元

编 委 会

前　言

植物品种的特异性（distinctness）、一致性（uniformity）和稳定性（stability），简称为DUS。DUS测试是指依据相应植物属（种）的测试技术与标准，通过田间种植试验或室内分析对待测试品种的DUS进行评价的过程。

我国于1999年4月23日加入国际植物新品种保护联盟UPOV，标志着植物新品种保护制度的正式实施。截至2015年12月31日，我国品种权申请量居UPOV成员第二位，仅农业部受理的申请达15 552件，授予品种权6 258件。为保证授权的客观、公正和科学，农业部于2001年在全国一级农业生态区成立了植物品种DUS测试机构，包括1个测试中心和14个测试分中心，2014年和2015年分别新建了4个分中心，现在分中心已达22家。DUS测试机构成立以来，共完成69个植物属（种）10 000多个品种测试、100多个品种真实性鉴定，DUS测试为植物品种的授权、市场监管和司法鉴定等提供了强有力的技术支撑。

2016年1月1日实施的《中华人民共和国种子法》规定审定、登记和保护的植物品种必须具备特异性、一致性和稳定性，首次明确了DUS测试的法律地位，使DUS测试在引领育种技术创新、种业管理等方面将发挥越来越重要的作用。

目前DUS测试体系团队共有人员159人。根据测试体系二期项目建设规划，到2020年，将新建15个测试分中心、改（扩）建1个测试中心和14个测试分中心，挂牌建立28个多年生无性繁殖植物新品种测试站，DUS测试人员和测试品种数量必将大幅度增加。为加强植物品种测试技术研究和人才培养，2015年5月，农业部科技发展中心（农业部植物新品种测试中心）成立了测试技术专业委员会，下设信息技术、生物技术、大田作物、蔬菜、观赏植物和果树6个技术组。为充分发挥各技术组的作用，鼓励DUS测试体系年轻人在扎实做好DUS测试工作的同时，加强测试技术的学习、研究与交流，促进优秀人才脱颖而出，打造一支与国际接轨、专业技术过硬的审查测试人员队伍，农业部科技发展中心在各测试分中心支持下，收集、梳理了自测试体系成立以来至2015年12月31日15年间，测试人员和管理人员以第一作者发表的DUS测试技术研究论文146篇。由于汇编篇幅限制，这些论文交由DUS测试技术委员会评选后，仅对评选出的44篇优秀论文列入汇编。

本汇编论文研究内容全面，涉及大田作物、蔬菜、观赏植物和果树等植物品种的表

型测试技术、分子测试技术和信息技术等方面，吸收和借鉴了国外DUS测试技术最新成果，并结合我国DUS测试实际情况，实用性强，对从事DUS测试技术研究以及新入职的DUS测试人员具有较高参考价值。

本汇编论文是测试体系成立以来，对国内外DUS测试技术经验的总结和探索，凝结了测试体系全体人员的智慧和汗水，在此向大家表示感谢。同时，由于时间仓促，难免存在错误疏漏之处，欢迎大家批评指正。此外，随着DUS测试技术的发展和测试方法的改进，希望大家再接再厉，继续对DUS测试技术进行深入研究，提高我国DUS测试技术和水平，为现代种业发展做出新的贡献。

编委会

2016年3月23日

目　录

测试技术研究

植物品种特异性、一致性和稳定性测试15周年论文集

Impact of Light-induced Changes in Anthocyanin Coloration Intensity on DUS Test

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Abstract: Intensity of anthocyanin coloration is a frequently-used characteristics in DUS tests, and its expressions are susceptible to the change of light intensity. Based on the requirement that characteristics used in DUS tests should be consistent and repeatable in a particular environment, research related to the impact of light-induced changes in anthocyanin coloration intensity on DUS test is essential to reveal the problems of current DUS tests. Results gained in this study showed that plants of the same rice variety tested in the given environment had indentical expressions in the same characteristic of anthocyanin coloration intensity, indicating the characteristics were consistent; if plants were tested in environments with different light intensity, the characteristics had various expressions, especially the results of DUS test differed from each other. The fluctuation would bring great troubles to DUS test, and it would also obviously lead to problems for harmonization of variety descriptions. This study proposed approaches for this situation, including deleting characteristics of anthocyanin coloration intensity, making the growing conditions fixed, or making DUS tests of the same plant genera or species concentrated.

Keywords: DUS test; light intensity; intensity of anthocyanin coloration; characteristics

1 Introduction

In recent years, more and more attention has been paid to intellectual property rights due to the increasing international cooperation and exchanges. Plant breeder's right (PBR), or plant variety protection (PVP) is one form of intellectual property rights. PBR has drawn public interest with the development of marketing of plant varieties [1,2]. To protect the legal rights of plant breeders, Chinese government has issued "Regulation of the People's Republic of China on the Protection of New Varieties of Plants" (hereinafter referred to as "Regulation") in 1997. China became the 39th member of International Union for the Protection of New Varieties of Plants (UPOV) in 1999, and accordingly, new varieties of plants can be applied for protection in China since then [3]. In accordance with the "Regulation", PBR is granted to the plant variety which can be distinguished (D) from any other variety commonly known when the application is filed, and which is considered as sufficiently uniform (U) and stable (S) [4,5]. It is noted in UPOV Convention that a variety is defined by its characteristics and therefore those characteristics are the basis for DUS [4,5]. Besides examining whether a variety meets DUS criteria, DUS test can also generate a description of variety. Each plant genera or species has an exclusive set of characteristics in corresponding DUS test guideline [6]. These characteristics are generally morphological characteristics, e.g., plant height, plant width, or physiological characteristics, e.g., growing period [6,7]. The range of expression of each characteristic in the test guidelines is divided into a number of states for the benefit of description, and

原载于：Biomedical Engineering and Environmental Engineering: 21 - 27.

the wording of each state is attributed a numerical "Note" [4]. Characteristics can be grouped as qualitative characteristics, quantitative characteristics, and pseudo-qualitative characteristics based on their different expression ways. Qualitative characteristics are expressed in discontinuous ways, and these states are self-explanatory and independently meaningful [e. g. , sex of plant: dioecious female (1), dioecious male (2), monoecious unisexual (3), monoecious hermaphrodite (4)] . Expressions of quantitative characteristics differ from one extreme to the other, and such differences are continuous, and thus their expression can be recorded on a linear scale [e. g. , width of leaf: very narrow (1), narrow (3), medium (5), wide (7), very wide (9)] [4,5]. As a rule, qualitative characteristics are usually independent from environment, while quantitative characteristics are often polygenically inherited and can be influenced by environmental conditions [8-10].

Anthocyanin coloration is one of the most common characteristic frequently used in DUS test. Above 85% DUS test guidelines adopt one or more characteristic (s) of anthocyanin coloration [11]. Anthocyanin coloration can appear in different parts of plant, e. g. , root, stem, leaf, flower, fruit, seed, etc. Characteristics of anthocyanin coloration include two kinds: absence or presence of anthocyanin coloration, and intensity of anthocyanin coloration. The first kind is generally described as "plant part: anthocyanin coloration", and the states include "absent" (Note 1) and "present" (Note 9) . This kind of characteristics is absolutely qualitative characteristics. Description for the second kind is "plant part: intensity of anthocyanin coloration. " This kind of characteristics is quantitative characteristic, and the states include "very weak (1), weak (3), medium (5), strong (7), very strong (9) . " The states can also be "very weak (1), weak (2), medium (3), strong (4), very strong (5)" or "weak (1), medium (2), strong (3)" if the span of intensity fluctuation is small.

Intensity of anthocyanin coloration can be influenced by cultivation conditions, especially light and nutrient, e. g. , growth hormone NAA, carbohydrate [12-14]. Light plays an important role in the formation of anthocyanin. Strong light can induce more accumulation of anthocyanin [12,13]. According to requirements of DUS test, any characteristic listed in DUS Test Guideline should be sufficiently consistent and repeatable in a particular environment [4]. Since characteristics related to anthocyanin coloration are frequently used in DUS test, and intensity of anthocyanin coloration can be influenced by light changes, it is of great importance to research the impact of light-induced changes in anthocyanin coloration intensity on DUS test. Studies on the influence of light changes to the changes of anthocyanin coloration intensity are involved in lots of papers. In contrast, report related to the impact of those changes on DUS test is yet unavailable.

This study conducts research on rice, the most important crop with the largest application and authorization in China. In DUS test of rice, there are eight characteristics related to intensity of anthocyanin coloration. Based on the "Guideline for the Conduct of Tests for Distinctness, Uniformity and Stability—Rice" (hereinafter referred to as "Guideline") [15], the impact of light-induced changes in anthocyanin coloration intensity on DUS test was analyzed here.

2 Subject and Methods

Two rice varieties were selected in this study. They were Liheizimi and Yunlu 99, marked as A1 and A2, respectively. Both varieties are typical representatives of anthocyanin coloration.

The test was carried out in 2009 and 2011. It began in April and ended in October. It was conducted in Yunnan Academy of Agriculture Science. Seeds were sown in trays for wet seedling nursing in April. One month later, the four-leaved seedlings were transferred to 30-cm-size pots. Four plants were grown in one pot.

After transferring, plants of the two varieties were grown in three conditions with different light intensity. The three conditions were marked as B1 (greenhouse condition), B2 (shelter condition), and B3 (outdoor condition) . Light intensity of the three conditions increases in sequence. Three repeats were set for each treatment.

DUS test was carried out on the eight characteristics of anthocyanin coloration intensity in strict accordance with "Guideline. " The eight characteristics are "leaf sheath: intensity of anthocyanin coloration," "lemma: intensity of anthocyanin coloration of keel (early observation)," "lemma: intensity of anthocyanin coloration of area below apex (early observation)," "lemma: intensity of anthocyanin coloration of apex (early observation)," "stem: intensity of anthocyanin coloration of nodes," "lemma: intensity of anthocyanin coloration of keel (late observation)," "lemma: intensity of anthocyanin coloration of area below apex (late observation)," "lemma: intensity of anthocyanin coloration of apex (late observation)", respectively. The eight characteristics were marked from C1 to C8 in order. Observations were recorded, and each expression state was attributed a numerical "Note".

Table 1 Treatments

Variety	B1 (Greenhouse)	B2 (Shelter)	B3 (Outdoor)
A1 (Liheizimi)	A1B1	A1B2	A1B3
A2 (Yunlu99)	A2B1	A2B2	A2B3

3 Results

The observations of the eight characteristics were translated into corresponding "Note". The "Note" was used to judge the expression states of the eight characteristics of the same variety under the above mentioned environments. The results indicate that plants of three repeats of the same treatment had the same expression in the same characteristic. In contrast, for the same variety, the same characteristic had different expressions in environments with different light intensity (Table 2). Changes in light intensity were positively correlated with changes in anthocyanin coloration intensity. Environment of B3 had stronger light than B1 and B2, and expressions of anthocyanin coloration intensity in B3 were the strongest in each characteristics. Figures 1 and 2 also show that intensity of anthocyanin coloration increased from left to right, or from B1 to B3. Characteristic expressions differed greatly (Table 2, Fig. 1 and 2). Taking characteristic of "lemma: intensity of anthocyanin coloration of keel (late observation)" (C6) as an example, expression of Liheizimi (A1) in B1 was very weak (Note1), while expression in B3 was very strong (Note 9) (Table 2 and Fig. 2).

Table 2 DUS test of the eight characteristics

Characteristics	Expressions and Notes					
	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3
C1	vw (3)	vw (3)	w (5)	vw (1)	vw (1)	w (3)
C2	w (3)	m (5)	s (7)	vw (1)	vw (1)	m (5)
C3	w (3)	m (5)	vs (9)	vw (1)	w (3)	s (7)
C4	s (7)	s (7)	vs (9)	s (7)	s (7)	vs (9)
C5	m (5)	m (5)	s (7)	vw (1)	vw (1)	w (3)
C6	vw (1)	m (5)	vs (9)	w (3)	w (3)	m (5)
C7	m (5)	s (7)	vs (9)	vw (1)	w (3)	s (7)
C8	s (7)	vs (9)	vs (9)	s (7)	vs (9)	vs (9)

(1) C1 - C8 are "leaf sheath: intensity of anthocyanin coloration," "lemma: intensity of anthocyanin coloration of keel (early observation)," "lemma: intensity of anthocyanin coloration of area below apex (early observation)," "lemma: intensity of anthocyanin coloration of apex (early observation)," "stem: intensity of anthocyanin coloration of nodes," "lemma: intensity of anthocyanin coloration of keel (late observation)," "lemma: intensity of anthocyanin coloration of area below apex (late observation)," "lemma: intensity of anthocyanin coloration of apex (late observation)", respectively.

(2) In "expressions and Notes," figures between brackets are Notes; outside brackets, vw means very weak, w: weak, m: media, s: strong, vs: very strong.

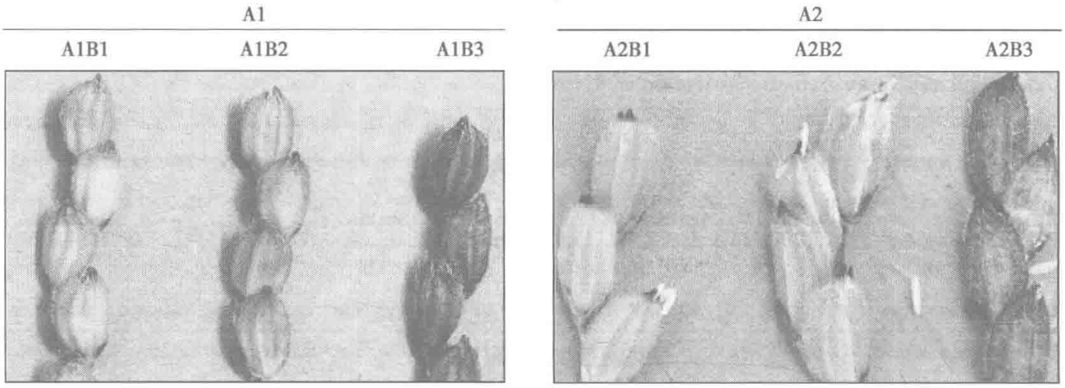

Fig. 1　Anthocyanin coloration intensity of lemma (early observation)

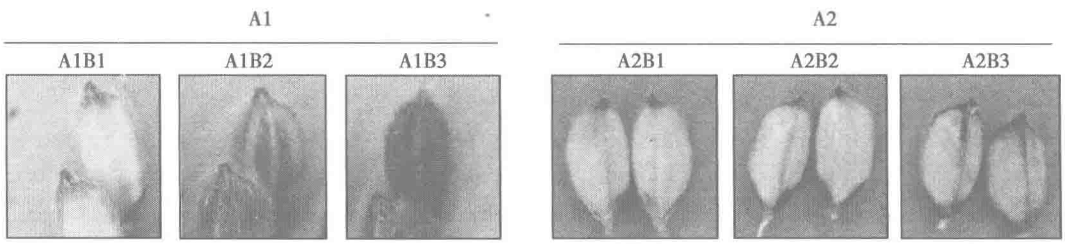

Fig. 2　Anthocyanin coloration intensity of lemma (late observation)

4　Discussion

Difference in light intensity will cause fluctuation in the intensity of anthocyanin coloration, and thus has impact on DUS test. Besides light, other factors, e. g. , nutrients can also have impact on accumulation of anthocyanin [14]. For field crop, natural light is difficult to control compared with other factors, and that is why light was chosen as a variable here.

DUS test is the basis for the granting of plant breeder's right. Any characteristic used in DUS test should be sufficiently consistent and repeatable in a particular environment [4,5]. In this study, results from three repeats of one treatment were the same, indicating the eight characteristics of anthocyanin coloration intensity were consistent in a given environment. However, results of the same characteristic from the same variety in environments with different light intensity were various. The fluctuation would bring great troubles to DUS test, and it would also obviously lead to problems for harmonization of variety descriptions. In China, there are 14 stations responsible for the DUS tests of different plant genera or species. The 14 stations are located in different ecological regions, and have different light intensity. Therefore, the same variety tested in different DUS test stations may have different results, and the results of different varieties of the same plant genera or species tested in different DUS test stations are not comparable with each other. To solve these problems, three approaches are proposed here: ①Characteristics of anthocyanin coloration intensity could be deleted in the DUS test guidelines of field crops. ②Growing condition with fixed light intensity and other environment factors can be used for certain plant genera or species, e. g. horticulture crops, vegetables, etc. ③For field crops, all the varieties of the same crop can be tested in one fixed DUS test station to avoid the absence of comparability of results gained in different stations.

>>> Acknowledgment

This study was assisted by Special Fund for Agro-scientific Research in the Public Interest of Ministry of Agriculture, China (200903008 - 14) .

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Sensitivity of Panicle Related Quantitative Characteristics of Rice DUS Test to Environmental Changes

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Abstract: Panicle related characteristics of rice have close relationship with rice yield. Among the 48 panicle related characteristics of rice DUS test, 8 quantitative characteristics were used to research whether environmental changes had impact on their expression. Results showed that, ①in general, measurements of the 8 characteristics of the same variety in different environments were visibly different, and when measurements were translated into "notes" according to "DUS Test Guideline of Rice", such difference could be narrowed, indicating the 8 characteristics were sensitive to environmental changes, while corresponding expression states and "notes" could weaken such influence; ②from the results of SPSS statistic analysis, environment factor were less influential than variety factor to the 8 characteristics; ③compared with previous research, the 8 panicle related quantitative characteristics were less affected by environmental changes than quantitative characteristics of vegetative growing period; ④ "notes" difference of the same variety in different environments were correlated with significance analysis of environment impact on characteristic expression, revealing the potential of statistic analysis in checking the availability of results of DUS tests gained from different test locations. To reduce the impact of environmental changes to characteristic expression, suggestion of fixing the DUS test of the same plant genus and species in one appointed station was also made in this study.

Keywords: DUS test; rice; panicle related characteristics; environment

1 Introduction

Panicle related characteristics of rice are research highlights and attracts great attention worldwide due to their direct bearing on rice yield [1,2]. Panicle related characteristics include quantitative characteristics, e. g. length or width of grain, qualitative characteristics, e. g. phenol reaction of lemma, and pseudo-qualitative characteristics, e. g. colour of glume [3]. Generally, quantitative characteristics are sensitive to environment changes [4-6]. That means, expression of quantitative characteristics can be different in various growing condition. Instead, qualitative characteristics are relatively stable and are less affected by environmental fluctuation [4-6].

Panicle related characteristics of rice are also important characteristics in rice DUS test. Among 78 characteristics of rice DUS test, 48 are panicle related characteristics, accounting for 62% [3]. DUS test is to check whether a bred variety is a new variety in following three aspects: whether the said variety (candidate variety) is distinct (D) in one or more characteristics from any other varieties known or used in market; whether plants of the said variety are uniform (U) in every characteristics; whether characteristics of the said variety are stable (S) after continuous growing cycles [7,8]. Both uniformity and stability are to test the homogeneity of morphological traits of the said variety, where uniformity empha-

原载于：Advomced Materials Research，2014，1028：368－373.

sized the homogeneity among individual plant in the same time, and stability emphasizes the homogeneity among populations in different growing cycles [8]. Obviously, DUS test is the precondition of the authorization of Plant Breeders Right (PBR) where the variety is applied for protection as a new variety [9,10]. Besides, DUS test can establish description for each variety, and provide basis for the exchanges of variety information, especially, the construction of information database of plant variety[11,12].

Rice is the most important food crop and has the largest application for PBR in China[4,13]. This study took rice as the materials, and panicle related quantitative characteristics as the target to research the variation of characteristic expression in environments with difference of temperature and light intensity, and thus, to explore the sensitivity of panicle characteristics to environmental changes.

2 Subject & Methods

Rice varieties of Chugeng 27 (A1), Diantun 502 (A2) and Yunlu 29 (A3) were chosen to conduct this study. The three varieties were bred in Yunnan, and has been used for years in Southern China. Chugeng 27 is suitable to be grown in areas with altitude of 1500 - 1850, and its growing period can last 170 - 175 days. Diantun 502 can be grown in areas with altitude of less than 1500, and its growing period is about 155 days. Among the three varieties, Diantun 502 has the shortest growing period and adapts to warm whether, while Yunlu 29 has the longest growing period and adapts to cool environment, and Chugeng 27 is in between.

Instead of paddy field, the trials were carried out in the form of pot planting, and thus, treatments in different environments (greenhouse, shelter and outdoor) were easy to be achieved. In 2009 and 2011, grains of the three varieties were sown in trays for wet seedling nursing in April. After one-month nursing, four-leaved seedlings were transferred to pots with 30 cm diameter, and each pot could hold 4 plants. Plants were treated in three different environments, namely outdoor (B1), shelter (B2) and greenhouse (B3) (Table 1) . The three environments were different in temperature and light intensity. On average, B1 was around 28. 2℃ from 8:00 to 18:00, B2 was 25. 5℃, and B3 was 33. 2℃. For each treatment of each variety, 3 repeats were set to avoid random error. Aside from temperature and light intensity, other growing conditions were the same, including irrigation, fertilization, disease or pest control.

Table 1 Treatments

Variety	Outdoor (B1)	Shelter (B2)	Greenhouse (B3)
Chugeng27 (A1)	A1B1	A1B1	A1B1
Diantun502 (A2)	A2B1	A2B1	A2B1
Yunlu29 (A3)	A3B1	A3B1	A3B1

DUS test were carried out in accordance with "DUS Test Guideline of Rice" (hereinafter referred to as "Guideline") . 8 quantitative characteristics, which are panicle related, were measured (Table 2) . Each characteristic was measured in 20 plants of a variety. The measurements were given corresponding "notes" based on the ranges of expression states of each characteristic (Table 3) . Expression ranges the 8 characteristics are all divided into 9 states. Taking characteristic of "panicle: length" for example, expression states for the "1 - 9" notes can be described as "very short (Note 1), very short to short (Note 2), short (Note 3), short to medium (Note 4), medium (Note 5), medium to long (Note 6), long (Note 7), long to very long (Note 8), very long (Note 9)" . SPSS, a commonly used statistics software, was also applied to conduct further analysis.

Table 2 8 panicle related quantitative characteristics

No.	Name of Characteristics	Range of Note
C45	panicle: length	1～9
C59	panicle: number of grains	1～9