Legume vigour

Zorica Nikolić1, Zlatica Miladinov1, Sanja Vasiljević1, Snežana Katanski1, Gordana Tamindžić2, Dragana Milošević2, Gordana Petrović2

1Centre of Excellence for Legumes, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, 21000 Novi Sad, Serbia
2Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksima Gorkog 30, 21000 Novi Sad, Serbia
*Corresponding author: zorica.nikolic@ifvcns.ns.ac.rs

Received 7 December 2020; Accepted 19 March 2021

ABSTRACT

Seed vitality and vigour are the two most common parameters related to seed quality. It is crucial to have reliable methods and tests for seed quality and seed vigour testing. The standard germination test can be used to predict field emergence, but laboratory seed testing conditions are often in conflict with field conditions. Validated tests for vigour evaluation in legumes are the conductivity test, the accelerated ageing test and the tetrazolium test. Also, other types of vigour tests have been used to solve different problems in the seed sector. The modern approach, the computerised image analysis of legume seeds and sprouts, based on interactive and traditional methods, is a promising alternative for vigour determination.

Keywords: seed quality, vigour, legumes, tests.

ИЗВОД

Виталност семена и вигор су два најчешћа параметра у вези са квалитетом семена. Веома је нажно имати последице методе и тестова за испитивање квалитета и вигора семена. Стандардни тест књижевност се може користити за предвиђање вигора у пољу, али услови у којима се излазе семе током испитивања често су у супротности са условима на пољу. Валидоване тестове за процену вигора легуминоза су тест проводљивости, тест убрзаног старења и тетразолијум тест. У процесу испитивања животне способности семена легуминоза зависно од проблема који би требало решити, користе се и други вигор тестови. Савремени приступ, заснован на интерактивним и традиционалним методама машинског учења, омогућава поуздану оцену квалитета семена и представља добру альтернативу за објективно одређивање вигора.

Кључне речи: квалитет семена, вигор, легуминозе, тестови.

1. The legume family

The abbreviated list of cultivated species contains 352 species from 55 families (Egli, 1998), but most of our food supply is based on several plant species. According to the total mass of food produced, the most important group includes cereals, primarily wheat, rice and maize, the production of which is higher than all other cereals combined (Mirić, 2016). After cereals, legumes are the second most widespread crops. Compared to cereals, they have a high lysine content and are important as supplements to cereals in predominantly vegetarian diets (Lešić, 2003).

The legume family (Fabaceae Lindl.), the third largest family of flowering plants, includes 3 subfamilies with 800 genera and 20,000 species that are distributed worldwide (Lewis et al., 2005). The variability within the family is great, with different forms of plants ranging from annual and perennial herbaceous plants to woody forms and vines. Also, there is variability in the shape and size of the seeds, ranging from 1 mm seeds of the Australian legume species Pycnospora lutescens to 18 cm seeds of the coastal tree Mora oleifera (Gunn, 1981).

There are 39 genera with about 250 species in the flora of Serbia, distributed in different habitat types (Diklić, 1972). This family of plants includes many economically important plants, some of which are horticultural plants, and a significant number of which are honey and medicinal plants.

In Serbia, bean (Phaseolus vulgaris L.), pea (Pisum sativum L.) and soybean (Glycine max L. Merr.) are the most widely grown for human consumption, while broad bean (Vicia faba L.), chickpea (Cicer arietinum L.), lentil (Lens culinaris Medik.), grass pea (Lathyrus sativus L.), lupine (Lupinus spp.) and black-eye pea (Vigna unguiculata L.) are grown to a lesser extent. Alfalfa (Medicago sativa L.) and red clover (Trifolium pratense L.) are the most important perennial forage legumes used as feeds for farm animals in Serbia (Dokić et al., 2019).
2. Seed vigour

Organised seed testing started more than a hundred years ago to avoid unscrupulous practices prevalent in seed trade during the nineteenth century. The first laboratory for seed testing was established in Tharandt, Saxony, in Germany, in 1869 under the direction of Friedrich Nobbe. Seed testing spread rapidly in Europe during the following twenty to thirty years. At the beginning of the twentieth century (1900), about 130 seed testing stations were operating in Europe. In the United States, the first seed testing laboratory was opened in 1876. In India, the first seed testing station was established in 1961. Determination of seed quality and its viability indicates which seed lots can be placed on the market, and therefore it is very important to have reliable methods and tests for seed quality and seed vigour testing. No modern technique that could be used in the routine analysis of seed germination has been established yet. Standard tests with numerous shortcomings are still being used, and they often do not show realistic seed behaviour under field conditions. The standard germination test is an indicator of seed quality, which can be used to predict field emergence, if soil conditions are nearly ideal (Durrant and Gummerson, 1990). However, the circumstances under which the seed is observed during the analysis frequently disagree with field conditions. Different vigour tests are used to obtain more precise information about seed lot quality (Milošević et al., 1996).

Seed vitality and vigour are the two most common parameters related to seed quality. The vitality of seeds is defined as the ability of seeds to germinate and give normal seedlings; it shows metabolic activity in seeds, indicates the activity of enzymes catalysing the reaction needed for germination and seedling growth (Delouche and Caldwell, 1960).

Seed vigour, the most important trait that determines the quality of a seed lot, occurs during seed development and is the product of the interaction between genotypes and the conditions in which the seed is produced (Lekić, 2001). Healthy and mature seeds retain their vigour during different periods of time depending on plant species, storage conditions and seed history. The entire seed production is based on the principles of maintaining and improving seed vigour. Seed viability in the broadest sense reflects: 1) germination rate, 2) rate of growth and rooting under field conditions, 3) general effect of endosperm reserve substances in relation to seedling growth (Whalley et al., 1966). Seed vigour is the sum of traits that gives the seed the potential for rapid, uniform germination and development of normal seedlings under different field conditions (AOSA, 2002). It reflects the ability of the seed to form normal seedlings under unfavourable growing conditions that can occur in the field. Seed vigour is not an individually measurable trait, such as germination, but a term that describes several characteristics related to the following aspects of seed lot condition: rate and uniformity of seed germination and seedling growth, seed germination under adverse environmental conditions, seed condition after storage, primarily retention of germination capacity (ISTA, 2020). Similarly, Sun et al. (2007) defined seed vigour as a comprehensive property that can be measured by the determination of seedling length, root length, seedling fresh mass, seedling dry mass, germination, seed longevity and tolerance to adverse conditions. These parameters have a quantitative nature and are affected by conditions during seed maturation, harvesting and storage. For now, seed vigour is only a concept, rather than a set of specific characteristics of seeds or seed lots. The value obtained by vigour determination is affected by the genetic constitution of the seed, the environmental conditions under which the mother plant is grown, nutrition applied during that period, seed maturity at harvest, seed weight and size, mechanical injuries, ageing, pathogens etc. (Milošević et al., 2010).

3. The application of vigour tests

Vigour tests do not serve to predict the exact number of seedlings that will grow and survive in the field although many vigour tests give results similar to field emergence. Vigour tests are commonly used by seed companies to monitor seed quality during different stages of production and processing (Woodsock, 1976).

The examination of seed viability provides information about the sowing value of seeds for a wide range of environmental conditions, as well as about the germination and storage potential of seed lots. In this way, we obtain additional data, which, in addition to the standard examination of seed germination, enable the separation of seed lots of acceptable germination (Marcos Filco, 2015). Seed lots that have similar germination values may differ in their tendency to ageing and decay, i.e. in seed response to storage and field conditions (Jovičić et al., 2013). A vital seed lot is the one with the ability to achieve good results, even under environmental conditions that are not optimal for the species. Seed with good physiological quality is characterised by high germination and longevity, slow decomposition of reserve substances and pronounced vigour. Quality seed gives strong and well-developed plants under a broad range of conditions. Lowered physiological quality of seed results in the decreased survival of plants in the field under adverse and stressful conditions, leading to variations in crop density (Finch-Savage and Bassel, 2016).

High vigour seed gives crops that are uniform in terms of germination, plants that have more leaf area and dry mass, and crops with a better productivity index (Kolchinski et al., 2005). Seeds with high vigour have better germination capacity, higher reserve capacity, and more soluble proteins, starch and soluble sugars (Henning et al., 2010). Seeds with reduced vigour often give robust plants in order to establish reduced intraspecific competition within the population, accompanied with a decrease in population density.

Vigour tests are understandable if there is a problem with emergence or seed ageing during storage. It was noted that some soybean cultivars, as well as lots with high germination, behave differently during emergence under field conditions (Hyatt et al., 2007). Methods used for the evaluation of vigour can be direct or indirect. Direct methods are those tests for which unfavourable environmental conditions and other conditions are reproduced in the laboratory, and the percentage or rate of seedling emergence is recorded. Indirect tests measure other seed traits that have been shown to be related to some aspects of seedling behaviour.
4. Vigour tests

AOSA (2002) classifies vigour tests into three groups: (1) stress tests, which include the accelerated ageing test and the cold test, (2) tests for the evaluation of seedling growth, which include seedling vigour classification and the seedling growth test, (3) biochemical tests, which include the tetrazolium test and the conductivity test.

According to McDonald (1975), vigour tests are divided into:
1. Physical tests, which determine seed characteristics, such as: seed fractional composition, 1,000-seed weight, moisture content and swelling rate. These tests are cheap, fast and show a positive correlation with seed vigour.
2. Physiological (direct) tests, which use germination and seedling growth parameters. There are two types of these tests:
   - Tests in which germination occurs under favourable laboratory conditions (standard laboratory method, growth intensity test),
   - Tests in which the seed is exposed to adverse environmental conditions (cold test, accelerated ageing test and Hiltner test);
3. Biochemical (indirect) tests, which include the tetrazolium test and the conductivity test.

One type of vigour test cannot be applied to all plant species. ISTA validated tests for testing the viability of legume seeds are: conductivity test, accelerated ageing test and tetrazolium test.

5. Conductivity test for legume seeds

The electrical conductivity test was originally designed to explain why peas that achieve high germination under laboratory test conditions show reduced germination in the field. It has been determined that seeds with low vigour release substances, such as sugars or other metabolites into the soil solution. That can increase the activity of fungi, and thus hinder the development of seedlings, especially at lower temperatures (Matthews and Bradnock, 1967).

The conductivity test is based on immersing the seeds in deionised water and measuring the electrical conductivity of the water after 24 hours. Seed lots with strong electrolyte release, or more precisely with high conductivity, are considered weakly vigorous, while those with low release (low conductivity) are considered highly vigorous (ISTA, 2020). A significant part of the electrolyte released by the seed into the water consists of inorganic ions, and the total electrolyte concentration is indirectly measured by the conductivity test.

By determining the amount of substances released into the water in which the seed has been immersed, such as sugars, amino acids, organic acids, proteins and phenolic substances and inorganic ions K+, Ca2+, Mg2+, Na2+, we indirectly obtain data on the state and structure of cell membranes (Marcos Filho, 2015). The presence of dead seeds in the sample can affect the conductivity result; therefore, it is recommended to prepare a sample that excludes damaged seeds.

The conductivity test is applied as the standard test for vigour examination in chickpea (Cicer arietinum), soybean (Glycine max), bean (Phaseolus vulgaris) and pea (Pisum sativum, only garden pea), and refers to potential emergence in the field (ISTA, 2020).

Santos et al. (2007) discovered that brown seed coat of soybean can affect the results of the electrical conductivity test because of its higher lignin and protein content, which results in a lower rate of absorption and different vigour among genotypes.

In search of optimal conditions for chickpea conductivity test, Castilho et al. (2019) concluded that seed imbibition time can be reduced to 4 hours, and the best conditions in terms of the volume of water and temperature used to immerse chickpea seeds are 25 ml of water at a temperature of 30°C.

A similar experiment aimed at evaluating the effect of different temperatures combined with a shorter soybean seed imbibition time in the conductivity test was conducted by Catão and Caixeta (2019). The conductivity test was performed in 20 replicates with 10 seeds immersed in 25 ml of deionised water, and the imbibition time varied: 10, 30, 60 and 120 min at temperatures of 30°C, 35°C, 40°C, and 45°C. Identification of soybean seed lots with different vigour can be performed if the seed imbibition time is shortened to 30 min at 30°C or 35°C.

6. Accelerated ageing test

Seed viability can be checked by the accelerated ageing test. Its purpose is to provide additional information on the results of the germination test in such a way that atypical seeds that are likely to germinate well under field conditions are separated from seeds that will not germinate (Baskin, 1970). Accelerated ageing is a stress test where seeds are exposed to high temperature and high relative humidity (> 95%) for a short period of time. During the test, the seed absorbs moisture from the humid environment; therefore, the test increases the humidity of the seed at a high temperature, causing accelerated seed ageing. Seed lots that have high vigour will withstand these external stressful conditions, retain high germination and age more slowly than parties with weak vigour. In this test, the seed is exposed to high temperature and high relative humidity, which results in increased seed moisture and increased activity of hydrolytic enzymes (e.g. lipoxygenase), i.e. increased lipid peroxidation under stress conditions (McDonald, 1999). In addition, this test provides information on seed longevity (Te Krony, 2001).

Lipid peroxidation as well as decreased superoxide dismutase and peroxidase activities are particularly pronounced in soybean seeds when accelerated ageing is applied. The duration of the storage period, the type of storage and the characteristics of the soybean variety affected the degree of seed damage and the ability of seeds to resist the negative effects of ageing (Balešević-Tubić et al., 2011).

Various factors may lead to slower germination, formation of weak seedlings, and in some cases to loss of soybean seed viability. The most common are: high soil moisture, low soil and air temperature, crust, etc. An analysis of the effect of localities and climatic conditions on seed quality and viability in different soybean genotypes was conducted by Vujaković et al. (2011a), who showed that the quality and vigour of seeds were influenced by genotype and agro-ecological conditions in soybean seed production. The accelerated ageing test can be used to obtain data on the
physiological quality of seeds, predict germination in the field, and assess seedling vigour, or as an aid in the interpretation of soybean seed lot vigour (Matera et al., 2019).

Standard vigour tests are being developed and modified to find suitable tests for individual plant species. Dias et al. (2020) conducted a study in order to establish an effective methodology for performing an alternative vigour test with saturated NaCl salt solution (76% relative humidity) and determining the optimal period and temperature of chickpea exposure. In the saturated saline solutions test, the ‘BRS Aleppo’ cultivar showed consistent results at a temperature of 45°C for 24 hours, while the ‘Cicero’ cultivar had low correlation with the results of the standard accelerated ageing test, indicating that further research is needed to establish an appropriate methodology.

7. Tetrazolium test

The tetrazolium test was developed in 1950 as a test for obtaining rapid general estimates of seed viability, particularly in species in which dormancy was expressed, and in which the germination test would last too long (Hampton and TeKrony, 1995). The tetrazolium test plays an important role in the assessment of seed physiological quality, not only because of its relative speed, but also because of information about the vitality and vigour of the seed. In addition, it is possible to diagnose factors that affected seed quality, such as mechanical damage, insect damage, seed ageing during storage, and pre-harvest weather conditions (Franca-Neto and Krzyzanowski, 2019). The test indirectly measures the respiratory processes in the mitochondria of seed tissue cells. Reduction of a colourless solution of tetrazolium salt under the action of the enzyme dehydrogenase produces triphenylformazan, which is carmine red in colour. The red-coloured living parts of the seed are clearly distinguished from the dead parts of the seed. Detailed assessment of the differences in location, colour and intensity of staining allows an assessment of whether the seed is vital or non-vital.

A database containing information and visual characteristics of soybean seeds classified according to the types and intensity of damage obtained by the tetrazolium test was published by Pereira et al. (2019). The set of these data enables an efficient way of automatic definition of soybean seed vigour through machine learning and data collection methods, which can be useful for researchers, seed analysts and farmers.

The tetrazolium test needs to be adjusted to specific plant species. If a concentrated solution of tetrazolium that intensely stains the seed is used in the test, this prevents the distinction between the types of seed injuries. Paraíso et al. (2019) analysed three chickpea genotypes at three combinations of imbibition time and temperature (41°C for 4 hours, 41°C for 6 hours and 30°C for 18 hours) and two concentrations of tetrazolium solution (0.1% and 0.5%) in order to determine the most suitable conditions for performing the tetrazolium test. Clearly coloured chickpea seeds are obtained when 0.1% tetrazolium solution is used, either at a temperature of 41°C for a shorter time (4 or 6 hours) or at a lower temperature of 30°C for a longer time (18 hours). Based on the visualisation of seed damage according to vigour and seed vitality, the seed can be divided into four classes.

8. Other vigour tests

In the study of legume viability, depending on the problem that needs to be solved, other vigour tests have been used. The cold test proved to be suitable for assessing the effect of fungicide application, selecting cultivars and seed lots suitable for early sowing, assessing physiological damage caused by prolonged storage under adverse conditions, and frost or drought damage, and for measuring the impact of mechanical damage on germination in cold and moist soil (AOSA, 2002).

The test is based on the exposure of the seed to combined stress conditions, low temperature and pathogens. A major disadvantage of the cold test is variability in the results due to the use of different soil types. Nijestein and Kruse (2000) noted that the least variation in results was obtained when sand was used as a substrate.

Testing the viability of soybean seeds under optimal conditions provides information on the maximum possible germination and initial growth. As field conditions are rarely optimal, it is necessary to perform testing under conditions that are close to environmental conditions in order to obtain a more reliable forecast of crop behaviour in the initial stages of growth and development (Srebrič et al., 2010). Germination of soybean seeds in sand under cold test conditions was lower than optimal conditions by 7-22%, depending on genotype. In treatments where soil was used as a substrate, germination values were even lower. The best germination was achieved on chernozem, and the germination rate was affected more by the substrate (sand and soil) than by temperature. The intensity of the initial growth (determined by the length of root and hypocotyl) decreased significantly under unfavourable temperature and soil conditions, and the number of sprouted plants and their growth decreased at a lower temperature.

In their research on the effect of soybean maturity group on the physiological quality of soybean seed, Carvalho et al. (2017) analysed 16 genotypes of different maturity groups using vigour tests: accelerated ageing test, cold test, conductivity test, and tetrazolium test. Some of super early soybean genotypes had good physiological indicators of seed quality, while other cultivars had low quality. It was concluded that maturity group did not affect the physiological quality of soybean seed, although very early cultivars had high vigour, viability and germination capacity.

Recently, a study has been conducted on the use of various vigour tests for chickpea seed in order to determine correlation with field germination and identify the most suitable test for predicting seed behaviour under field conditions (Emaeilzade-Moridani et al., 2011). The standard germination test did not correlate well with field germination, but the vigour tests had higher correlations at all three sowing dates, compared to the standard germination test. Tests which proved to be the most suitable vigour tests were as follows: conductivity test, accelerated ageing (41°C for 48, 72, 96 and 120 h) test and growth intensity test.
8.1. Factors affecting seed vigour

Seed viability is a complex property defined during different stages of seed development. It is affected by genetic and environmental factors. A very comprehensive overview of the factors influencing vigour and seed quality was given by Finch-Savage and Bassel in their paper (2016).

![Figure 1. Factors influencing vigour and seed quality (from Finch-Savage and Bassel, 2016)](image)

Seed production under conditions of water stress, insufficient quantity of nutrients, and extreme temperatures often results in the development of poor vigour seed (Castillo et al., 1994). Also, mechanical damage during harvesting and processing, as well as improper storage can adversely affect vigour (Peksen et al., 2004).

Genetic factors, such as hardness, disease resistance and chemical composition have a favourable effect on seed vigour, and the determination of seed viability is also used as an indicator of the storage potential of seed lots (AOSA, 2002).

Fast and uniform germination in the field is of great importance for achieving high yields while ensuring good seed quality in cultivated plants. The vigour test is more sensitive than the standard germination test and it is a good choice for the determination of seed lot quality under field conditions. Seed lots that do not differ in germination percentage may differ in terms of seed ageing and seed behaviour under field conditions (Kolasińska et al., 2000).

Low vigour seeds resulted in soybean yields with number of pods and number of seeds per pod inversely correlated with yield, i.e. seed vigour had a direct effect on plant population and an indirect effect on seed yield (Caverzan et al., 2018). It was shown that high temperature and humidity significantly reduced soybean seed viability, which caused a serious reduction in crop density and yield. High temperatures, which lead to seed damage, and later to reduced germination, encourage an increased leakage of electrolytes and macromolecules. Such damage can be due to the direct influence of high temperatures on membrane integrity (Lekić, 2003). Vujaković et al. (2011a) examined germination and viability using vigour tests (accelerated ageing test, Hiltner test and cold test) in soybean seeds produced under conditions of dry farming and irrigation. The seed produced under irrigation conditions had higher germination, while significant differences in seed viability were found between the tested varieties and lines.

The most common vetch species grown in the production area of Serbia are common vetch (Vicia sativa L.), hairy vetch (Vicia villosa Roth), and Hungarian vetch (Vicia pannonica Crantz). The most significant issue in the production of vetch seed is lodging, which can reduce seed yield and quality. Vujaković et al. (2011) conducted research on vetch seed viability using the standard laboratory test, cold test, Hiltner test and three variants of the accelerated ageing test (39°C, 72 h; 39°C, 96 h; 45°C, 48 h). Unfavourable conditions during vigour testing led to a reduction in the seed germination of common vetch. The growth parameters of the aboveground part of the seedling were the lowest while using the Hiltner test, and the length and biomass of the root system were the lowest using the accelerated ageing test. The lowest values for all tested parameters in hairy vetch and Hungarian vetch were obtained using the Hiltner test. Different variants of the accelerated ageing test showed that the examined parameters did not depend on the temperature applied, but that the duration of temperature affected vetch seed viability.

Seed vigour can be used as the most reliable indicator of the storage potential of soybean and sunflower seeds, compared to the standard germination test (Balešević-Tubić et al., 2010, 2011).

The accelerated ageing of seeds, i.e. exposure of the seed lot to high temperatures and relative humidity leads to loss of strength, and ultimately to loss of vitality. During accelerated ageing, the seed is characterised by loss of germination, reduced germination rate and poor seedling growth (Tatić et al., 2008).

Negative correlations between seed viability and seed moisture content in red clover were obtained at storage temperatures of 30 to 65°C (Ellis and Hong, 2006). Red clover and alfalfa seeds stored under hermetic conditions for 14.5 years at different moisture contents (2–15%) and temperatures (-20, 30, 40, 50 and 65°C) retained vitality. Red clover and alfalfa seeds preserved their viability at a temperature of -20°C and at a moisture content of 2.2–14.9% and 2–12%, respectively.

Plant breeding has made great progress in improving crops. However, it is a very time consuming process, and the genetic resources associated with vigour are limited. In natural conditions, it is very difficult to find highly vigorous seeds. With the development of modern approaches, such as genomics, transcriptomics and proteomics, numerous potential candidate genes and proteins involved in seed vigour have been found. In order to improve seed vigour or yield in soybean, some of the potential candidate genes that can be used in the breeding programmes Tu1, Tu2,
9. Development of software programs for determining seed viability

The need to improve the vigour analysis procedure has led to new computer programs; new specific systems have been developed to assess the viability of seeds of different species. SVIS® Vigour Determination Program developed by Sako et al. (2001) for lettuce seeds as a model system has been successfully adapted to seeds of different species. SVIS® Vigour Determination systems have been developed to assess the viability of seeds as subjective to some extent (Liu et al., 2015). The SVIS analysis of the bean seed lot vigour test, which was conducted by Gomes Junior et al. (2014), gave reliable and similar results to the standard laboratory test. In order to further standardise the method, it was found that similar results were obtained if the seedlings were analysed three or four days after germination. Laboratories usually use methods to visually assess the quality of soybean seeds, which include the assessment of seedlings. However, these methods are time consuming and usually destructive, and they can be subjective to some extent (Liu et al., 2015). The modern approach to the classification of soybean seeds and sprouts is based on the interactive and traditional methods of machine learning. Interactive machine learning (IML) is one of the recent and promising approaches defined by algorithms that can communicate with both computational and human agents (Holzinger, 2016). With timely and repeated use of human knowledge and skills, IML approaches defined by algorithms that can communicate with both computational and human agents (Holzinger, 2016). With timely and repeated use of human knowledge and skills, IML approaches can be effective in problems with small or complex data sets when traditional machine learning methods become ineffective (Holzinger, 2019). The combination of these machine learning algorithms with computer vision has brought new and promising perspectives for seed quality analysis (Lin et al., 2019).

The high precision of the models developed on the basis of interactive and traditional machine learning enables reliable classification of soybean seeds according to their appearance, as well as fast and non-subjective determination of soybean seed vigour (de Medeiros et al., 2020).

Acknowledgment

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-9/2021-14/200032*.

Declarations of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References


