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Research Note

Morphological and Physiological Changes of *Katuray* (*Sesbania grandiflora*) during Seed Maturation

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***Katuray* (*Sesbania grandiflora*), whose flowers are consumed as steamed vegetables, is an important green manure crop in the Philippines. Understanding different processes leading to seed maturity can aid in determining *S. grandiflora's* seed quality, preventing premature harvesting for seed increase, ensuring efficient propagation and production of seedlings, and breeding new varieties of this crop. Multiple flowers were tagged, and three to five pods were collected five days after anthesis (DAA) and at a five-day interval thereafter until 100% germination was achieved. Seed size, moisture content (MC), dry weight, germination percentage, and embryo development were observed and recorded. Seed length was highest at 55 DAA and decreased thereafter. Moisture content had an increasing trend from 15 to 50 DAA then decreased afterwards. The increase in seed size can be attributed to the increase in accumulated dry matter while the decrease in MC can be attributed to the weakening ability of the seed as a sink and its acquisition of desiccation tolerance. Germination started at 45 DAA and reached 100% at 60 DAA. For embryo development, walking stick stage was observed at 55 DAA and full seed maturity was attained at 75 DAA. Seed physiological maturity was observed at 60 DAA, where highest seed dry weight was measured. Correlational analysis of different seed parameters showed a positive correlation of seed size, dry weight, and germination rate while a negative correlation was observed in germination rate and moisture content. Documentation of the morphological changes during seed maturity and the relationship of these changes have biological, breeding, and production implications in seed systems for this underutilized crop species.**

Keywords: embryo, maturation, morpho-physiology, seed maturity, *sesban*, seed physiology

INTRODUCTION

Seeds come in diverse sizes and shapes. The variation occurs from one family to another and even at the species level (Salgotra and Chauhan 2023). These changes are not limited to the size and shape of seeds but can also be seen on the pattern of development and seed maturation (Sripathy and Groot 2023). Annual seeds have a faster rate of development compared with biennials and perennials. After fertilization, the seed starts to develop, and morphological changes can be observed through the development of the embryo, endosperm, and seed coat as the seed matures. Chemical and biochemical changes, on the other hand, can be observed through the seed's physiological characteristics such as embryogenesis, development of cotyledon, increase in germinability, size and changes in moisture content (Gupta et al. 2022).

Different crop species have different seed characteristics and maturation patterns. Therefore, the changes and patterns of maturity, germination, and even seed vigor varies (Obura and Lamo 2024). This was reported in different crops like onion (*Allium cepa*) (Ramya et al. 2012), faba bean (*Vicia faba*) (Ghassemi-Golezani and Hosseinzadeh-Mahootchy 2009), eggplant (*Solanum melongena*) (Passam et al. 2010), and proso millet (*Panicum miliaceum*) (Ragupathi et al. 2017) among others. Variation was also detected even across cultivars of some crops like bell pepper, sweet pepper (*Capsicum* sp.), and eggplant as reported by Ghasemnezhad et al. (2011), Howard et al. (2000), and Botey et al. (2021).

Marcos-Filho (2022) stated that seed maturation is used to determine phenological differences among species and that seed size is one of several parameters of seed maturity that is used to determine these phenological differences. Being able to determine, understand, and document seed development and their maturation patterns are essential to produce high-quality seeds. Without prior knowledge of the phenology and maturation patterns of seeds, harvesting prior to physiological maturity may produce low quality or non-viable seeds due to immature embryo, too high moisture content, or too low dry matter.

Chanda et al. (2017) stated that seed size, as one of the quality parameters in seed traits, was a determinant of germinability and vigor of *Sesbania* species. Accumulation of food reserves, i.e., proteins, oils, lipids, carbohydrates, and even water results in an increase in seed size (Bewley et al. 2013). The seed becomes a strong sink as it develops for assimilates and its dry weight (DW) increases (Liu et al. 2016). Seed size generally increases as the seed matures but decreases after the seed has attained maximum DW due to water loss. Seed moisture, the measure of the water content of the seed, also changes as the seed matures. Since moisture or water is the transporter of assimilates, an increase in moisture content (MC) is expected as the seed matures. On the other hand, as the seed starts to weaken as an assimilate sink, the moisture also decreases. As the cotyledon develops, assimilates accumulate inside as the seed's food reserves.

Sesbania grandiflora, locally called *katuray*, is one of the important green manure crops in the Philippines. It is a leguminous tree whose flowers are eaten as steamed vegetables (Ladha and Bell n.d.) The dried methanolic extract of *S. grandiflora* is known to have anti-diabetic and anti-hypertensive effects. Although it is underutilized, it has many promising benefits, which contributes to the increasing demand for *Sesbania* in urban communities. On the other hand, the propagation of this species is not well studied, and its breeding is also not being considered. Thus, research on its seed development will provide information about its phenology and maturation. This will help establish the seed's physiological maturity and the appropriate time to harvest for multiplication. Furthermore, correlational analysis of seed dry weight, seed size, and germination rate can help determine the relationships between these traits and their relation to days to maturity. This study provides a deeper understanding of seed development and offers a foundation for creating models to explore the seed development process in *katuray*.

The study aimed to determine the physiological maturity of *S. grandiflora* through the morphological and physiological changes that occurred during seed maturation. These changes were measured through seed size, DW, germination, MC, and embryo development. Correlations were also observed for all the parameters measured, and their relation to days to maturity was also determined.

Materials and Methods

2.1 Plant Material Used

The standing *katuray* (*S. grandiflora*) trees (14.16025, 121.24613) behind the Agriculture Systems Institute of the College of Agriculture and Food Science, University of the Philippines Los Baños were used in the study. Three trees were present but only two were chosen since both have red flowers and the other has white. Since genotype differences may affect the data as it may cause variation other than the days from maturation, the red-flowered trees were used.

Several flowers of the same age were initially tagged, and pods were collected 5 d after anthesis and every 5 d thereafter. The seeds were extracted immediately after pod collection at the preparation laboratory of the Institute of Plant Breeding, College of Agriculture and Food Science, UPLB. The study was conducted from the 2nd week of September to the 3rd week of November 2019.

2.2 Parameters Collected and Analysis

Morphological and physiological data of *S. grandiflor*a seeds collected were seed size, DW, MC, and germination. Seeds in the middle of the pod were the only ones collected and used, as the position of the seed may influence all parameters measured. Three replications were made with 10 seeds per replicate. The average and slope were computed to check the rate of increase or decrease of each parameter and data on seed size, DW, MC and germination were plotted using Microsoft Word Office 365.

Seed size (cm). Size was measured as seed length using a vernier caliper. The mean was computed and used in plotting the graph.

Seed dry weight (DW) (g). Ten seeds were used in the measurement of size per collection/sampling time for a total of 150 seeds. The seeds were placed in pre-measured glassine bag (1.25 x 4.0 in), sealed with a paper pin, and oven dried at 70ºC for 3 d. After 3 d, the seeds were allowed to cool inside a desiccator with dried silica gel for 4 h. Seed DW was computed using the formula:

Seed DW = (Total DW – weight of vessel) / No. of seeds

Seed MC (%). Ten seeds per replicate at three replications for a total of 30 seeds were collected per sampling time. Seed MC was determined using low constant oven method. The MC was computed using the formula:

$$\% MC = \frac{(M2 - M3)}{(M2 - M1)} \times 100$$

where: M1 = weight in grams of the container and its cover
M2 = weight in grams of the container, its cover and its contents before drying
M3 = weight in grams of the container, its cover and its contents after drying

Germination (%). Three replicates of 10 seeds each were germinated and observed after 7 d, then the number of seeds that germinated were counted. For the purpose of this study, a 2 mm protrusion of radicle from the seed coat was considered as germinated. The percent germination was computed using the formula:

$$\% G = \frac{\text{number of germinated seeds}}{\text{total number of seeds sown}} \times 100$$

Seed development. Two seeds were dissected per sampling time. Seeds were cut longitudinally and viewed using a Celestron digital handheld microscope. The image was then captured and processed in Microsoft Powerpoint office 365.

Correlations of different parameters to days to maturation were computed using the rcorr function from the Hmisc package of the R software (Harrell and Dupont 2019). This function calculates the correlation matrix for the data. The rcorr function from the Hmisc package computes pairwise correlation coefficients, the number of observations, and the *p*-values for testing the hypothesis of correlation. Correlation was presented in correlogram.

RESULTS AND DISCUSSION

Morphological Changes During Seed Maturation of *S. grandiflora*

Growth

S. grandiflora generally blooms in September. Anthesis occurs from morning up to 1:00 pm. After the flowers have been fertilized, the petals continuously dried up then abscised from the flower and only the pods remained.

The pod matured at 70 d after anthesis (DAA) where drying starts from the distal end of the pod going up to the point of attachment. Flowers are commonly ephemeral, i.e., last for 1 d only. At 5 DAA, the pod was observable, and a slow rate of filling was observed from then on. Since *S. grandiflora* is a perennial, it has a longer maturation compared to the other members of the Fabaceae family, e.g., mung bean (*Vigna radiata*) and soybean (*Glycine max*), where maturity can be observed even at 18 DAA.

Seed Size

Changes in seed morphological characters such as size and DW of *S. grandiflora* from 5 to 75 DAA are shown in Fig. 1. Seed size, as represented by length (cm), increased as shown by the positive slope from 15 to 50 DAA (Table 1). The seed length started to decrease from 55 DAA as shown by the negative slope of -0.808. The decrease in seed length can be attributed to the decrease in water content which is collaborated by the decrease in MC from 50 to 75 DAA (Fig. 1). A small increase in the slope of seed length at 60 DAA can be attributed to DW accumulation. High DW means that the seed is physiologically mature and starts its independence from the mother plant. As the seed becomes independent of the mother plant, the connection of the plant to the pod starts to dry up due to an increase in ethylene and ABA activity (Bareke 2018; Syamsuwida and Aminah 2020). Consequently, the seed weakens to be the sink, exhibiting a decrease and eventual termination of both water and nutrient uptake, thus also decreasing in length or seed size (Sripathy and Groot 2023).

Size is an important quality trait in seeds (Adebisi et al. 2013) since it affects germination, nutrient content, and seedling emergence, among other factors (Kaydan and Yagmur 2008). The seeds used in this study were almost uniform in size at 60 DAA at approximately 2.5 – 3.2 cm. In a study on *Sesban* species where seed length was approximately 3.703 mm, Orwa et al. (2009) stated that the seeds exhibited varying sizes, germination percentages, and levels of vigor.

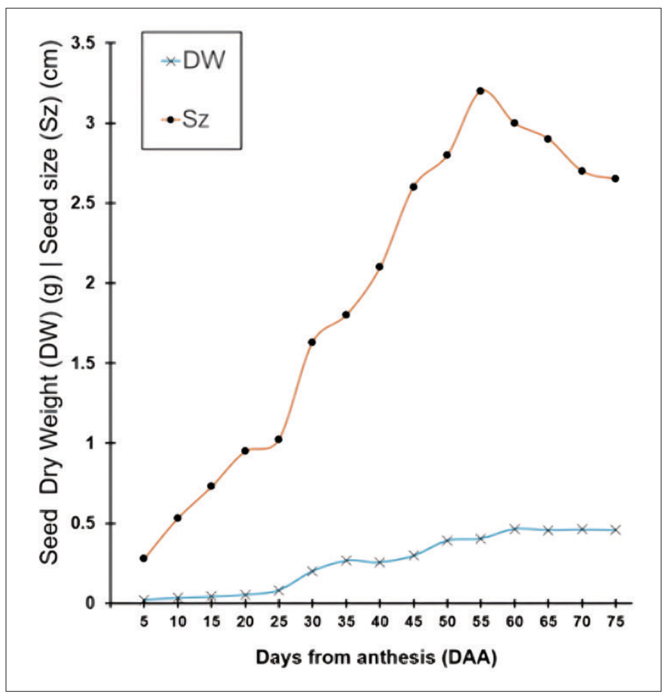

Fig. 1. Changes in morphological characters of *S. grandiflora* seeds such as seed size and dry weight (DW) from 5 to 75 days after anthesis (DAA).

Dry Weight (DW)

At 5 DAA, the DW was observed to be 0.021g. Increasing DW was observed from 5 to 35 DAA but the slope shows that the rate of increase every 5 d varied (Table 1). There was a slight decrease in DW at 40 DAA then it increased until 60 DAA, where the highest seed DW was attained (Fig. 1). Seed DW was observed to plateau between 60 – 70 DAA.

Seed development begins with the increase in weight due to nutrient accumulation and water uptake (Marcos-Filho 2022). During the first days of development, seed filling is slow, as was observed in *S. grandiflora*, where there was a very low increment on the rate of increase per 5-d interval (Table 1). Over time, there was a faster rate of accretion which can be attributed to the continuous dry matter accumulation and uptake as the seed, on its peak of development, becomes a strong sink. As the seed attains the highest DW, it starts to become independent of the mother plant and the seed maturity phase begins. Highest seed DW indicates physiological maturity (Marcos-Filho 2022). In addition to this, the highest seed DW was attained at 60 DAA after the seed started to decrease in water content as shown by MC (Fig. 2).

Physiological changes during seed maturation of *Katuray*

Moisture Content (MC)

Increasing MC was observed from 15 to 50 DAA as shown by the positive slope (Table 1) with the highest MC obtained at 50 DAA (Fig. 2). It decreased after 50 DAA since the slope changed from 1.2 to -0.8 at 55 DAA. Seed physiological maturity was attained at 60 DAA when DW was at its highest (Fig. 1). From 50 DAA onwards, a negative slope can be observed which implies a decrease in MC (Table 1).

Table 1. Computed slopes of seed size, dry weight (DW), and moisture content (MC) of S. grandiflora from 5 to 75 DAA.

DAA	Slope		
	Seed Size	Dry Weight	Moisture Content
5	0.0000	0.0042	0.0
10	0.0000	0.0026	0.0
15	3.8599	0.0018	3.9
20	0.2909	0.0018	0.3
25	1.2688	0.0059	1.3
30	1.0173	0.0238	1.0
35	0.0030	0.0131	0.0
40	1.9781	-0.0021	2.0
45	0.6140	0.0085	0.6
50	1.2240	0.0187	1.2
55	-0.8080	0.0026	-0.8
60	-4.4160	0.0118	-4.4
65	-2.0583	-0.0014	-2.1
70	-0.7572	0.0011	-0.8
75	-2.2165	-0.0003	-2.2

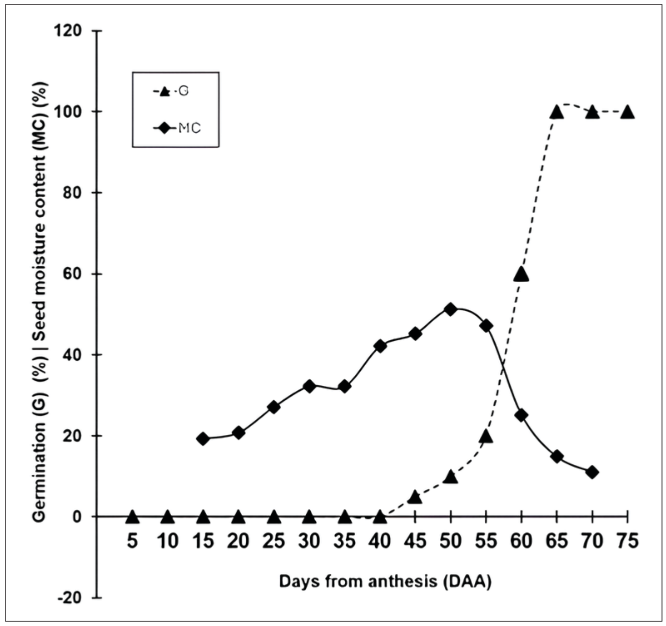

Fig. 2. Changes in physiological characters of *S. grandiflora* seeds such as germination (G) and moisture content (MC) from 5 to 75 d after anthesis (DAA).

Santos et al. (2020) have observed that in the case of okra (*Abelmoschus esculentus*), the highest accumulation of dry matter occurred while MC was also high (Dos Santos et al. 2018). Specifically, high-water content of 56.6% was observed at 30 DAA. In seed development, water has an important role in the assimilation of solutes like minerals and photosynthates from source to sink (Iqbal et al. 2016). Thus, water is vital for the dry matter accumulation after assimilation (Wang et al. 2021). After a certain period, there is a decrease in MC when the seed enters maturity, then maturation drying occurs when it has accumulated enough assimilates (Bewley et al. 2013).

Germination Percentage

Germination test of *S. grandiflor*a seeds harvested at different time intervals starting at 5 DAA showed that radicle protrusion and emergence were observed in 5% of the seeds at 45 DAA. This increased to 10% at 50 DAA and up to 20% at 55 DAA, then further increased to 60% at 60 DAA and eventually achieved 100% germination at 65 DAA. The germination percentage of maturing seeds varies from one species to another and increments as the seed matures (Marcos-Filho 2022). For instance, Santos et al. (2020) tagged flowers of okra cv. Sta. Cruz and harvested fruits from 5 to 65 DAA and reported that maximum seed germination and seedling emergence were observed at 50 DAA. Furthermore, no germination was observed in seeds from 25 DAA. This means that at 30 d, germination was completed in okra.

Physiological maturity in seeds is characterized by the highest germination rate in combination with the highest accumulation of DW. In this regard, this should have been the optimal stage for harvesting, but since it also coincides with high moisture content of seeds, harvesting at physiological maturity is less ideal. Hence, harvesting should be delayed for a few days especially if no efficient drying facility is available. Premature harvesting leads to losses due to low viability and low vigor while very late harvest results in shattering, reduced seed vigor and increased susceptibility to pests and fungal and bacterial diseases (Bewley et al. 2013). Despite the clear advantages of harvesting at physiological maturity, this practice is not universally adopted due to limited information on the specific physiological maturity stages of various crops. Consequently, there is a need for comprehensive research and dissemination of information on physiological maturity to optimize the most appropriate time to harvest and enhance crop yield and quality.

Seed Development

The development of *S. grandiflora* seeds, cut longitudinally and viewed using a Celestron digital handheld microscope, is shown in Fig. 3. Differences in the color of the embryo and their size were also observed through the photomicrograph. Noticeable changes of embryo growth can be seen from 20 DAA until the embryo attained the walking stick shape at 55 DAA. The walking stick stage is the last stage of embryogenesis where shoot and root apical meristems are also receptive to development. The high MC during embryo development (Fig. 2) was exhibited by the shiny green appearance of the seed up to 55 DAA (Fig. 3). At 60 DAA, the embryo exhibited a lighter color (Fig. 3) when the MC decreased (Fig. 2). From 65 to 75 DAA, the seeds were drier compared with those from earlier days of observation.

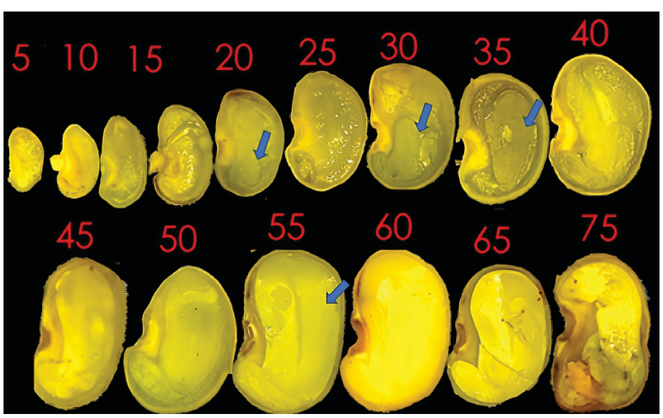

Fig. 3. Dissected seeds of *S. grandiflora* from 5 to 75 DAA as observed through a Celestron digital handheld microscope. Blue arrows indicate changes in the embryo.

Embryogenesis in plants commences through an asymmetrical cell division where two cells, the apical cell and the basal cell, develop, with the basal cell as the larger one. At this point, the polarity of the embryo is established with most of the plant embryo rising from the apical cell. As the basal cell becomes the suspensor, it serves as a conduit of nutrients for the developing embryo. Further development gives rise to the globular stage of embryogenesis (Gilbert 2000).

At this stage, the embryo becomes globular (Day 20, Fig. 3), then further divides, losing its globular shape and becoming heart-shaped, the curvatures of which are the preliminaries of the cotyledon (Day 30, Fig. 3). This will further divide to develop the torpedo or the walking stick stage where the suspensor degenerates and the apical meristems of both the shoot and the root develop. The meristem will be the starting point of the different organs. Embryogenesis halts as the embryo attains its torpedo or walking stick stage, followed by desiccation, and then germination when the conducive conditions are met.

Correlation Among Different Seed Characteristics in Relation to Days to Maturity

Morphological changes in the fruit and even in the seed are factors that dictate or even affect seed quality. Seed development and maturation must be determined and their patterns documented as these relate to seed germination and vigor. This has production implications as this information is vital for knowing the best time for harvesting seeds with the highest quality characteristics (Obura and Lamo 2024). In this study, relationships across different selected seed morphological and physiological characteristics and days to maturity were determined using correlational analysis. Fig. 4 shows the correlation coefficient for all pairs of variables measured. In terms of days to maturity and DW and between DW and seed size, a significant, very strong positive correlation was observed. Furthermore, strong positive correlation was observed between germination and DW and between germination and days to maturity. However, only moderate positive correlation was observed between seed size and germination and a significant negative moderate correlation was observed between germination and MC.

A direct relationship between seed DW and seed size was observed in *Sesbania*. This agrees with the report of Kadapi et al. (2018) in maize (*Zea mays*) where seed size was positively correlated with seed weight. In the same study, they observed that seed DW was positively correlated with germination. The positive correlation of seed size and weight to germination rate may be attributed to higher energy sources of heavier and bigger seeds than small seeds (Zhang et al. 2012). Kadapi et al. (2012) further discussed that bigger seeds had higher germination because bigger seeds have higher food reserves. The amount of food reserves affects the germinability of the crops as observed in field corn, wheat (*Triticum*), and even in *Artocarpus* species (Sadjad et al. 1993; Nik et al. 2011; Yusuf et al. 2014). Furthermore, an inverse relationship was observed between germination and MC. This inverse relationship is expected in developing seeds—as seeds mature or near the end of maturation, seeds naturally undergo desiccation which significantly reduces moisture content. While moisture content is declining as seeds mature, this coincides with increasing germination because this is also the point when the seeds have already acquired all the nutrients it needs, as indicated by the maximum dry weight.

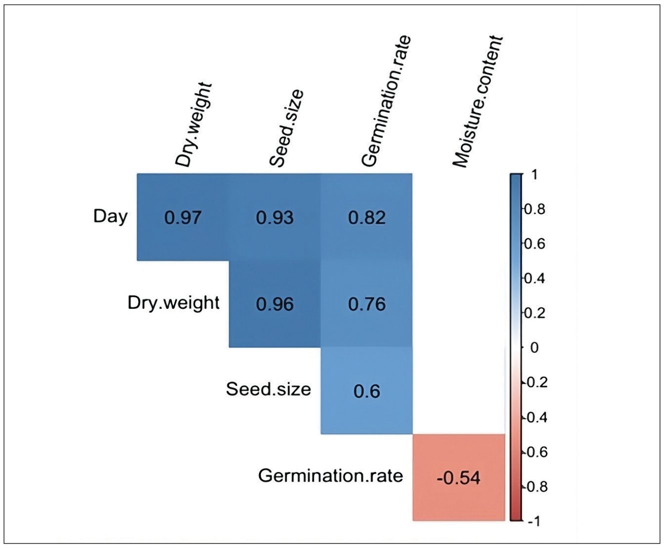

Fig. 4. The correlogram shows correlation coefficients for all *katuray* seed-related variables, with more intense colors for more extreme correlations, and correlations not significantly different from 0 as represented by a white box.

CONCLUSION

Seed maturity in terms of changes in the morphophysiological characters of *Sesbania grandiflora* was investigated. Seed size, dry weight (DW), moisture content (MC), and germination were observed. Seed physiological maturity was established to be at 60 DAA, where the highest seed DW was observed. At 55 DAA, walking stick stage of embryo development was observed. Moisture content increased from 15 to 50 DAA and started to decrease thereafter. Seed size was highest at 55 DAA and decreased thereafter. The increase in DW was due to dry matter accumulation while the decrease in water content was attributed to the natural desiccation of seeds where vascular connections between the mother and the seed become nonfunctional. Furthermore, correlational analysis of seed parameters revealed positive correlation among seed size, DW, and germination, while negative correlation was observed between germination and MC. The correlational information and the data observed have biological, breeding, and production implications for seed systems for this underutilized indigenous crop.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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