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RESEARCH ARTICLE

Evaluation of Recombinant Inbred Lines of *Oryza glaberrima* and Improved Samba Mahsuri for Antixenosis and Tolerance against *Nilaparvata lugens* (Stal)

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Abstract

The selected recombinant inbred lines (RILs) of *Oryza glaberrima*, improved Samba Mahsuri (ISM), resistant (Ptb33) and susceptible (TN1) checks were evaluated for antixenosis and tolerance against brown planthopper (BPH), *Nilaparvata lugens* under glasshouse conditions for two wet crop seasons of 2020 and 2021. The mean least number of nymphs were recorded on a resistant check, Ptb33 (7.04) followed by resistant parent, *O. glaberrima* acc. IR75870 (9.04), while highest nymphs were settled on the susceptible check, TN1 (15.63). IR75870 and homogenous resistant line (HRL) were least preferred by adult BPH males (3.25 and 3.40, respectively) and females (2.85 and 3.28, respectively) for settling. In tolerance studies of different germplasm lines against BPH, Ptb33, IR75870, and HRL, it took more time (19.40–23.00 days) to completely wilt. The mean lowest functional plant loss index (FPLI) was recorded from Ptb33 (19.28%), followed by IR75870 (21.32%) and HRL (21.77%), whereas the highest mean FPLI was recorded from TN1 (76.93%). A similar trend was observed among germplasm lines in case of plant dry weight loss index.

Keywords: *Oryza glaberrima*, Recombinant inbred lines, Brown planthopper, Brown planthopper adult settling, BPH nymph settling, Functional plant loss index, Plant dry weight loss index.

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Introduction

Rice is a staple food for a substantial portion of the world's population, particularly in East, South, and Southeast Asia, making it the second most consumed cereal grain. More than 90% of the world's rice is cultivated and consumed in Asia, which has 60% of the world's population. It is grown on around 154 million hectares per year, accounting for 11% of the world's cultivated land (Udayasree *et al*., 2021). Rice crop is attacked by more than a hundred insect species, of which 20 are economically important and brown planthopper (BPH); *Nilaparvata lugens* (Stal)) is one of them (Prakash *et al*., 2007). This phloem sap feeder is one of Asia's most destructive and notorious rice pests, capable of causing up to 60% yield loss under epidemic conditions (Roy *et al*., 2021). BPH nymphs and adults suck sap from the lower portion of the plant, resulting in yellowing of leaves, reduction in tiller number, plant height, and, finally, chaffy grains (Seo *et al*., 2009). Feeding also causes a reduction in chlorophyll and protein content of leaves followed by a reduced rate of photosynthesis, in case of severe attack, it causes extensive plant mortality referred to as the 'hopper burn' symptom (Kusumawati *et al*., 2018). BPH also acts as a vector and transmits rice grassy stunt virus (GSV) and ragged stunt virus (RSV) (Khush and Brar, 1991).

Among the various methods (cultural, use of resistant varieties, biological and chemical control) available for managing BPH at

farmers' fields. The most commonly adopted method is the use of insecticides and for many years, synthetic chemicals have been the mainstay of BPH control. As a result of the widespread and extensive use of pesticides, BPH has developed significant levels of resistance to several of the major types of insecticides (Zhang *et al*., 2016). Overall, BPH has evolved resistance to 29 compounds used in insecticide formulations (Sparks and Nauen, 2015), making chemical control one of the most insignificant approaches. Along with that, lack of proper planning and injudicious use of insecticides destroys the natural balance between BPH and natural enemies in rice agro-ecosystem (Sandhu and Sarao, 2020). Furthermore, it will also lead to increased production costs. Host plant resistance (HPR) is considered to be the most economical and eco-friendly strategy to manage the BPH. Therefore, a breeding programme for the development of BPH-resistant varieties with a different mode of host plant resistance is extremely important.

The exploration for the source of BPH resistance gene started back in 1967 and so far, more than 42 BPH resistance genes have been identified in Indica varieties, African cultivars, landraces and several species of wild rice germplasms (Kaur *et al*., 2022). The International Rice Research Institute (IRRI) has commercially released numerous BPH-resistant varieties for Asian farmers, but in recent years, these improved varieties have lost their resistance due to the emergence of new BPH biotypes. Therefore, incorporating new rice germplasm for identifying new resistance genes from novel sources against BPH in the breeding program will act as saviour against this most destructive pest of rice. So far, no BPH resistance gene have been designated and identified from *Oryza glaberrima* Steud. *O. glaberrima*, commonly known as African rice, is one of the two domesticated rice species and is tolerant to low phosphorus, acidic soils, drought and other biotic stresses such as African gall midge, nematodes and blast. Keeping this objective in mind, present experiments were conducted for the evaluation of recombinant inbred lines (RILs) of *O. glaberrima* as a novel source for antixenosis and tolerance resistance against *N. lugens*. The goal is to incorporate these resistant traits into pre-breeding programs for the development of varieties resistant to BPH.

Materials and Methods

The selected recombinant inbred lines (RILs) of *O. glaberrima* and Improved Samba Mahsuri (ISM) after screening against BPH has been evaluated for antixenosis and tolerance against *N. lugens* under glasshouse conditions in the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30°90′ latitude and 75°81′ longitude) for two wet crop seasons of 2020 and 2021.

Test Plants

The seeds of ISM, *O. glaberrima* (acc. IR75870), Ptb33 (resistant check), TN1 (susceptible check) and 260 F_5 recombinant inbred lines were received from the Indian Institute of Rice Research (formerly Directorate of Rice Research), Hyderabad, India. The selected homogeneous resistant and homogeneous susceptible recombinant lines along with parents and checks after screening against BPH were used for the studies. The pre-sprouted seeds of these lines were planted in either seed boxes or pots, depending on the specific experiment. The containers thoroughly filled with nutrient-rich, well-puddled soil were placed in a glasshouse designed to prevent the entry of any insect pests before exposure to BPH.

BPH Rearing

The 30 days old TN1 plants were used for the rearing of BPH population under glasshouse conditions at the Rice Research Laboratories, Department of Plant Breeding and Genetics, positioned at 30° 54′ N and 75° 48′ E at (28 ± 2°C), 75% ± 5% relative humidity and 14 hours light/10 hours dark photoperiod (Heinrichs *et al*., 1985). These TN1 plants were sown in earthen pots (20 cm diameter) filled with nutrient-rich, well-puddled soil and replaced with fresh ones after the complete wilting of plants owing to insect feeding. To prevent the BPH population from escaping, the earthen pots were placed in rearing cages (68 x 50 x 50 cm) covered with nylon mesh, sewn along the four edges of the frame, and connected at the top with rope.

Antixenosis Resistance

BPH nymph settling

The seeds of each test germplasm were sown in lines randomly in rows at a spacing of 3.5 cm in seed box (45 x 35 x 10 cm) containing nutrient-rich well-puddled soil (Photo 1). Each row had 10 seedlings and the experiment was repeated thrice. 2nd – 3rd instar BPH nymphs @ 10 nymphs per seedling were released on 30 days old seedlings (3–4 leaf stage). After 1st, 2nd and 3rd days of release, the number of settled nymphs was counted. The seedlings were disturbed after each count for reorientation of the hopper nymphs. Six rows were randomly assigned to each seed box such that each replicate had a distinct arrangement of the rows, and the settling behavior of the nymphs was recorded using an average of three replications.

BPH adult settling

The seeds of test germplasms were sown in small pots (10 cm diameter, 15 cm height) and kept in a water trough in a complete randomized design with five replications (a single potted plant represented one replicate). All plastic pots were placed inside a small net house (120 × 80 × 40 cm) in a completely randomized design. On 30 days old seedlings (3–4 leaf stage), 100 pairs of one day old macropterous adults were released under free choice test. The number of male and female adults settled on the tested lines were counted 4, 8, 12, 24, 48 and 96 hours after release. For the

reorientation of the insects, the seedlings were disturbed after each count.

Germplasm Tolerance to BPH

This study was carried out in a series of five replications to determine the tolerance of germplasm lines to BPH. Like previous experiments, 30 day-old seedlings of each germplasm line were covered with cylindrical mylar cages with well-ventilated windows (Photo 2). About 25, 2nd–3rd instar BPH nymphs were released on each tested germplasm seedling and allowed to feed. The plant wilting was recorded when they started drying. Simultaneously, plants that were unexposed to BPH were also maintained as a check. The experiment was terminated after 40 days of nymphs release and the number of plants that did not wilt at the end of study was recorded.

Upon seedling wilting, planthoppers were killed by spraying insecticide and dead BPH were collected and then oven dried for 48 hours at 55°C and weighed using a digital balance. Infested and uninfested seedlings were also collected at the same time washed properly to remove soil and oven-dried at 70°C for 72 hours and thereafter weighed. Functional plant loss index (FPLI, %) and plant dry weight loss index (PDWLI, mg) to BPH were calculated by using the formula of Panda and Heinrichs (1983) as given below:

$$\text{Functional Plant Loss Index (FPLI, \%)} = 1 - \frac{\text{Dry weight of infested plants}}{\text{Dry weight of uninfested plants}} \times 100$$

$$\text{Plant dry weight loss index (PDWLI, mg)} = \frac{\text{Dry weight of uninfested plant} - \text{Dry weight of infested plant}}{\text{Dry weight of BPH progeny on infested plant}}$$

Statistical Analysis

Data from different experiments were subjected to analysis of variance following statistical software SPSS v 20.0. Statistical analysis was done using complete randomized design (CRD). Means and standard errors were calculated for comparison purposes at 5% level of significance. The arc sine (√percentage) and square root transformations were used for different data before statistical analysis to meet the normalization requirement.

Results

Antixenosis Resistance

BPH nymph settling

The mean nymph settling among germplasm lines differed significantly during different data recording days during both study years. After one day of nymph release the settling differed significantly on tested germplasm lines with a minimum number of nymphs were recorded on Ptb33 and highest on TN1 ($F_{5,12}$ = 190.541, p ≤ 0.0001). Similarly, after two days of BPH release, nymph settling was lowest on Ptb33 followed by IR75870, whereas the maximum number of nymphs were observed on TN1 followed by Improved Samba Mahsuri (ISM) and homogenous susceptible line (HSL) ($F_{5,12}$ = 2261.00, p ≤ 0.0001). Likewise, after three days of release, least nymphs were recorded on Ptb33 and highest on TN1 ($F_{5,12}$ = 738.09, p ≤ 0.0001) (Figure 1). Overall, the mean number of nymphs settled 54.96% less on Ptb33, 42.16% less on resistant parent (IR75870) and 40.31% less on homogenous resistant line (HRL) than on TN1 ($F_{5,12}$ = 2067.00, p ≤ 0.0001) (Table 1).

BPH adult female settling

The mean adult female settling among germplasm lines differed significantly during different time durations of data recording during both the years of study. After 4 hours of release a significantly minimum number of female adults were recorded on Ptb33, whereas the highest number of female adults were recorded on TN1 followed by ISM and HSL ($F_{5,24}$ = 140.24, p ≤ 0.0001). A similar trend was observed after 8 hours of release ($F_{5,24}$ = 208.41, p ≤ 0.0001). After

Table 1: Antixenosis studies on selected rice germplasm lines against *N. lugens* (mean of 2020 and 2021)

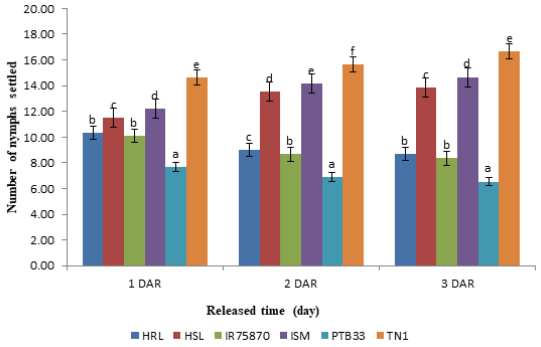
Germplasm line	*Mean number of settled nymphs# (Mean ± SE)*	*Mean number of settled adult female# (Mean ± SE)*	*Mean number of settled adult male# (Mean ± SE)*
Homogenous resistant line	9.33 ± 0.05^{c} (3.21)	3.28 ± 0.05^{c} (2.07)	3.40 ± 0.06^{b} (2.10)
Homogenous susceptible line	12.95 ± 0.11^{d} (3.73)	6.60 ± 0.04^{d} (2.75)	6.08 ± 0.05^{c} (2.66)
O. glaberrima (IR75870)	9.04 ± 0.02^{b} (3.17)	2.85 ± 0.07^{b} (1.96)	3.25 ± 0.06^{b} (2.06)
Improved Samba Mahsuri (ISM)	13.68 ± 0.06^{e} (3.83)	7.05 ± 0.07^{e} (2.84)	6.47 ± 0.04^{d} (2.73)
Ptb33	7.04 ± 0.06^{a} (2.84)	1.15 ± 0.07^{a} (1.47)	1.80 ± 0.08^{a} (1.67)
TN1	15.63 ± 0.10^{f} (4.08)	8.52 ± 0.03^{f} (3.08)	7.90 ± 0.18^{e} (2.98)
LSD (p = 0.05)	(0.03)	(0.04)	(0.05)

Figures in parentheses are the means of √n+1 transformations; Means within a column followed by the same letter are not significantly different at p≤ 0.05

Table 2: Tolerance studies on selected rice germplasm lines against *N. lugens* (mean of 2020 and 2021)

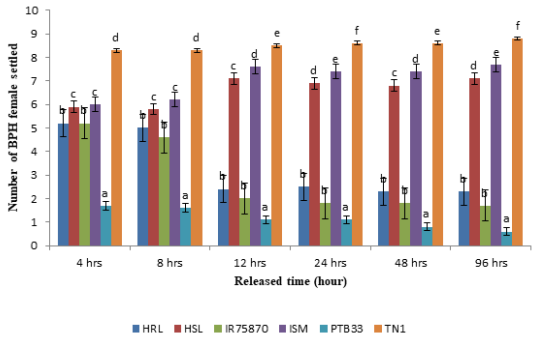
Germplasm line	*Days to wilt (days)#* *(Mean ± SE)*	*Functional plant loss index (%)** *(Mean ± SE)*	*Plant dry weight loss index (mg)#* *(Mean ± SE)*
Homogenous resistant line	19.40 ± 0.53^{b} (4.52)	21.77 ± 0.44^{b} (27.80)	17.38 ± 0.25^{b} (4.29)
Homogenous susceptible line	13.40 ± 0.19^{c} (3.79)	61.50 ± 0.56^{c} (51.63)	97.24 ± 0.25^{c} (9.91)
O. glaberrima (IR75870)	20.50 ± 0.63^{b} (4.63)	21.32 ± 0.57^{b} (27.48)	16.71 ± 0.17^{b} (4.21)
Improved Samba Mahsuri	12.70 ± 0.30^{c} (3.70)	66.15 ± 0.53^{d} (54.40)	104.93 ± 0.53^{d} (10.29)
Ptb33	23.00 ± 0.42^{a} (4.90)	19.28 ± 0.22^{a} (26.04)	14.40 ± 0.37^{a} (3.92)
TN1	10.60 ± 0.29^{d} (3.40)	76.93 ± 0.61^{e} (61.28)	126.74 ± 0.93^{e} (11.30)
LSD (p = 0.05)	(0.14)	(0.97)	(0.09)

Figures in parentheses are the means of √n+1 transformations; *Figures in parentheses are the means of arc sine √percentage transformations; Means within a column followed by the same letter are not significantly different at p≤ 0.05



DAR – Days after release; HRL – Homogenous resistant line; HSL – Homogenous susceptible line; ISM – Improved Samba Mahsuri; Different letters represent significant difference at the 0.05 level for each DAR separately; Bar represents the standard error

Figure 1: Number of *N. lugens* nymphs settled on rice germplasm lines (mean of 2020 & 2021)



HRL – Homogenous resistant line; HSL – Homogenous susceptible line; ISM –Improved Samba Mahsuri; Different letters represent significant difference at the 0.05 level for each DAR separately; Bar represents the standard error

Figure 2: Number of *N. lugens* females settled on rice germplam lines (mean of 2020 & 2021)

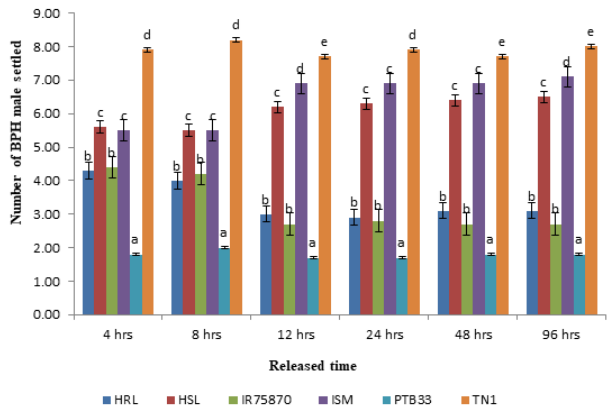
12 hours of release, least number of female adults were observed on Ptb33 followed by IR75870 and HRL whereas the maximum number of female adults were recorded on TN1 ($F_{5,24}$ = 463.79, p ≤ 0.0001). After 24 ($F_{5,24}$ = 494.29, p ≤ 0.0001), 48 ($F_{5,24}$ = 374.98, p ≤ 0.0001) and 96 ($F_{5,24}$ = 420.93 p ≤ 0.0001) hours of release, trend was similar as in the previous observations (Figure 2). Overall, the number of female adult settled 86.70% less on Ptb33, 66.71% less on IR75870 and 61.50% less on HRL than on TN1 ($F_{5,24}$ = 2469.00, p ≤ 0.0001) (Table 1).

BPH adult male settling

The mean adult male settling among germplasm lines differed significantly during different days of observations during both years of study. After 4 hours of release settling, adult males differed significantly and minimum number of male adults were recorded on Ptb33, the highest on TN1, followed by HSL ($F_{5,24}$ = 92.62, p ≤ 0.0001). A similar trend was observed after 8 hours of release ($F_{5,24}$ = 111.24, p ≤ 0.0001). After 12 hours of release, least number of male adults were observed on Ptb33 followed by IR75870 and HRL, whereas maximum on TN1 ($F_{5,24}$ = 196.80, p ≤ 0.0001). After 24 ($F_{5,24}$ = 114.77, p ≤ 0.0001), 48 ($F_{5,24}$ = 304.06, p ≤ 0.0001) and 96 ($F_{5,24}$ = 185.03, p ≤ 0.0001) hours of release, similar results were obtained (Figure 3). Overall, the number of male adult settled 77.22% less on Ptb33, 58.86% less on IR75870 and 56.96% less on HRL than on TN1 ($F_{5,24}$ = 658.92, p ≤ 0.0001) (Table 1).

Germplasm tolerance to BPH

The germplasm tolerance to BPH among germplasm lines differed significantly during both years of study. The resistant germplasm lines such as Ptb33, IR75870 and HRL took significantly more time to wilt as compared to susceptible germplasm lines like TN1, ISM and HSL ($F_{5,24}$ = 140.70, p ≤ 0.0001) (Table 2). Similarly, the functional plant loss index (FPLI) was lowest in Ptb33 followed by IR75870 and significantly differ from TN1 ($F_{5,24}$ = 2739.00, p ≤ 0.0001) (Table 2). Likewise, the plant dry weight loss index over



HRL – Homogenous resistant line; HSL – Homogenous susceptible line; ISM – Improved Samba Mahsuri; Different letters represent significant difference at the 0.05 level for each DAR separately; Bar represents the standard error

Figure 3: Number of *N. lugens* male settled on rice germplam lines (mean of 2020 & 2021)

the years differed significantly among the germplasm lines. It was least in Ptb33 followed by IR75870 and HRL ($F_{5,24}$=1135.00, p ≤ 0.0001) (Table 2).

Discussion

Host plant resistance (HPR) is an effective, economical, and eco-friendly method of pest management and is a core of integrated pest management programmes. The host choice test indicates the antixenotic factor, which is used to evaluate the germplasm. Different antixenosis studies have shown that BPH nymphs and adults tend to move to susceptible genotypes with thriving exposures. Therefore, it appears that antixenosis is preferably driven by feeding response because genotype with high phosphorus, potash and polyphenols are less preferred by planthopper (Mote and Shahane, 1994). The results of nymph and adult settling experiments are corroborated with the findings of Sable *et al*. (2014), Bhanu *et al*. (2014), Sarao and Bentur (2016), Sandhu and Sarao (2020) and Roy *et al*. (2021). Sarao and Bentur (2016) reported that nymph, adult female, and adult male settling was significantly lower on RP2068-18-3-5 and Ptb33 among nine genotypes tested. Similarly, Roy *et al*. (2021) evaluated 26 West Bengal rice landraces to study antixenosis resistance mechanism against BPH. Nymph settling was significantly less on Kalabhat, Ptb33 and Badshabhog than on susceptible check Swarna, whereas adult females settling was less on Ptb33, Janglijata and Gamra than on susceptible check Swarna.

Few QTLs or genes linked to different parameters of antixenotic resistance against BPH are reported in rice (Haliru *et al*., 2020). Kamolsukyunyong *et al*. (2013) reported that the sesquiterpene synthase gene in the rice cultivar Rathu Heenati affects the behavior of BPH during the first five days of BPH interaction with the rice plant. Prahalada *et al*. (2017) also reported that BPH nymph load was lowest on CR2711–76 harboring *Bph31* gene as compared to susceptible line Jaya and TN1, which possess no BPH-resistant gene.

In our studies we observed that among germplasm lines, Ptb33 harboring three genes such as *bph2+Bph3+Bph32* performed better than *O. glaberrima* (IR75870) and HRL in terms of nymphs settling, female settling as well as male settling. However, IR75870 and HRL are significantly at par with each other however in contrast, the susceptible check (TN1) and susceptible parent (ISM) with no resistance gene performed significantly poor. HRL performed far better than HSL as both are of F_5 generation and in this filial generation, almost 99% genetic homozygosity for desired traits will be achieved. Thus HRL showed similar results with that of the resistant parent (IR75870), whereas HSL showed results similar to that of the susceptible parent (ISM). Thus our findings demonstrated that BPH nymphs and adults less settled on IR75870 and HRL than on susceptible lines. Thus, IR75870, as a resistant parent, possesses an antixenosis mechanism which prevents BPH from settling on it, unlike ISM which BPH preferred.

Tolerance is the ability to grow and reproduce in spite of supporting a population approximately equal to that damaging a susceptible host (Painter, 1951). Panda and Heinrichs (1983) identified rice cultivars like Triveni, Kanchana and UtriRajapan with tolerance as a predominant component of BPH resistance through the elaborate development of the functional plant loss index (FPLI) method. Later, Alam and Cohen (1998) further refined the tolerance parameter as plant dry weight loss (PDWL) per unit dry weight of BPH gained. Similarly, Geethanjali *et al*. (2009) proposed a simple test of days to wilt for the tolerance parameter which is being readily accepted and is being followed by Ramesh *et al*. (2014) and Sarao and Bentur (2016). Roy *et al*. (2021) reported that under the continual pressure of BPH, days to wilt varied greatly between the test landraces. When compared to the susceptible check Swarna, the wilting of Badshabhog, Ptb33, and Kalabhat occurred substantially later. The link of genetic constituents with the tolerance component of plant resistance against BPH has already been confirmed by Qiu *et al*. (2014), who reported that the gene *bph7* in rice variety T12 majorly contribute to the resistance component of resistance against BPH.

Summary of tolerance studies showed that among the six tested rice germplasm lines, a new novel source from *O. glaberrima* (IR75870), Ptb33 and HRL possessed good tolerance against BPH. It is well established that the tolerant plant exerts less selection pressure on insect population and thus are useful for preventing the development of insect biotypes. It can be concluded from these experiments that settling and feeding of BPH on rice can be affected by the selection of a host plant. The restless behavior of BPH on resistant cultivars also makes them more vulnerable to natural enemies.

Conclusion

Finally, it is concluded that the accession IR75870 act as a novel source of *O. glaberrima* and HRL with high levels of antixenosis and tolerance against BPH. This will provide better opportunities for plant breeders and biotechnologists to develop resistant varieties from novel sources like *O. glaberrima* against BPH, which has AA genome as *O. sativa* L. also has good tillering and panicle bearing capacity. Spikelets are generally awnless and non-shattering. Our study shows that the development of a variety that can exhibit high levels of antixenosis and tolerance mechanisms against BPH could play a pivotal role in BPH management strategies.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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