Research article

The importance of understanding the distribution of GSTM1 and GSTT1 genotypes and haplotypes in a region with intense agriculture activity

Alessandro Arruda Alves, Fernanda Craveiro Franco, Fernanda Ribeiro Godoy, Jheneffer Sonara Aguiar Ramos, Hugo Freire Nunes, Thannya Nascimento Soares, Daniela de Melo e Silva

Brazil is one of the largest pesticide consumers in the world, mainly due to its intense agricultural activity. The State of Goias, situated in Central Brazil, is a region recognized as an essential producer of soy, corn, beans, sorghum, sugar cane, and cotton. In this study, we evaluated 602 unrelated individuals, distributed in central and southern regions of Goias, presenting combined frequencies (haplotypes) of the GSTT1 and GSTM1 genes. In all municipalities, the frequency of the GSTT1 null genotype was 38.2% and of the GSTM1 null genotype was 50.3%. Goiania, the capital of Goias, presented the highest frequencies of GSTT1 and GSTM1 null genotypes, probably due to a founder effect of non-representative colonizing ancestors. So, the ancestral population adapted to the environment, with the frequencies observed in Goiania. However, nowadays, as there is excessive use of pesticides, the community becomes susceptible to the harmful effects of xenobiotics exposure, mainly due to the high frequency of GSTT1 and GSTM1 null genotypes. As in Goias, the consumption of pesticides has shown considerable growth, haplotypes with null alleles are of high risk for the population. Our results indicated that it is essential to understand the frequencies of the GSTT1 and GSTM1 genes for the monitoring of risk groups, like farmers, who have contact with pesticides, directly or indirectly, as well as assisting in the development of preventive medicine practices.

1. Introduction

Brazil has been the largest consumer of pesticides in the world due to intense agricultural activity and government incentives (Abreu e Alonzo, 2014). Also, until October 2019, the Brazilian government authorized the commercialization of 382 pesticides, many of them banned in other parts of the world, making Brazil one of the countries that uses these products most, increasing the risk to human, animal and environmental health (Ministério da Agricultura, 2019).

The State of Goias is in Central Brazil and is one of the largest producers of corn, beans, cotton, sorghum, sugarcane, and soybeans in the country, with 75% of its exports composed of agricultural products (Instituto Mauro Borges, 2018). This vast production led to the use of 43,466.3 tons of active pesticide ingredients in 2017. Among the 10 (ten) most used in Goias are: glyphosate (15,486 tons of active ingredient [AI]); 2,4-D (4,026 tons AI); acephate (2,374 tons AI); mineral oil (1,949 tons AI); atrazine (1,919 tons of AI); mancozeb (1,797 tons of AI); cypermethrin (771 tons of AI); paraquat dichloride (718 tons of AI) and carbendazim (606 tons of AI) (IBAMA, 2019). In this case, Goias became the fourth-highest Brazilian state in commercialized pesticide use (Instituto Mauro Borges, 2018).

In this context, the use of pesticides has intensified, and there is an increase in inadequate handling and excessive use. These situations lead to the presence of pesticides in ponds, rivers, and many other water sources, polluting the environment and damaging both fauna and humans, mainly due to bioaccumulation in food chains (Souza et al., 2011). This may cause respiratory diseases (Ye et al., 2013), non-Hodgkin's lymphoma (Schinasi and Leon, 2014), lung cancer (Bonner et al., 2016), changes in reproductive hormones (Miranda-Contreras et al., 2013), Parkinson's disease (Panov et al., 2002), and Alzheimer's
In 2015, it was underreported that there were 31,900 cases of poisoning by pesticides in Goiás, according to the Minister of Health (Ministério da Saúde, 2016). In 2018, there were 12.74 incidences of poisoning notifications in every 100,000 inhabitants, while Brazil reported only 6.24 (Ministério da Saúde, 2018).

Pesticides are xenobiotics metabolized in two phases in the liver (Coles and Kadiubar, 2003). The superfamilly Glutathione S-transferase (GST) is involved in the conjugation phase, making the compounds less toxic and protecting the cell against oxidative damage and electrolyte substrates (Da Fonseca et al., 2010). The subclasses of the GST superfamilly studied most in mammals are the Mu (μ), Pi (π), and Theta genes. The gene GSTM1 (μ) is 4.2 kb, consisting of eight exons and is located on chromosome 1p13.3, with four known alleles. The most frequent is the wild-type allele, and the second one is the null allele characterized by a structural deletion of a region of the gene that causes the loss of the function of the enzyme (0) (Rodríguez et al., 2014; Strange et al., 2001).

The gene GSTT1 (π) is located on chromosome 22p11.2, is composed of six exons, and is flanked by two homologous regions HA3 and HA5. This gene has two alleles known as the most frequent allele (wild) and the null allele. This polymorphism occurs due to the recombination of the homologous regions HA3 and HA5, resulting in the deletion of 5.4 kb and, as a consequence, the loss of function of the enzyme (Rodríguez et al., 2014; Webb et al., 1996).

In this context, individuals with null GSTM1 genotypes are associated with an increased risk for the development of a variety of diseases, such as arteriosclerosis, Parkinson's disease, and various types of cancer, whereas the null GSTT1 genotype is associated with the colorectal and central nervous systems (Strange et al., 2001; Wahner et al., 2007; Wang et al., 2010).

Thus, the knowledge of the distribution of GSTM1 and GSTT1 polymorphisms in an agricultural population, such as that of Goiás, may assist in understanding the frequency of diseases related to poisoning by pesticides or other xenobiotic agents. As Goiás is a famous agricultural region, the study of these genes in municipalities of Central Brazil is significant. Therefore, it could be possible to associate the exposure to pesticides, with the null genotypes of GSTT1 and GSTM1, considered to be at risk for the development of chronic diseases, such as cancer.

2. Material and methods

2.1. Sample group

This research was approved by the Research Ethics Committee of the Pontifical Catholic University of Goiás, under the protocol number 1978/C19. To determine the power of our sample group, we used the following formula:

\[
\frac{z^2 + p (1-p)}{2p} \left( \frac{1}{N} \right)
\]

where N = population size; e = p-value; z = score z (1.96, considering a confidence interval of 95%). The population size of Goiás state is 6,000,000 individuals, according to the Brazilian Institute of Geography and Statistics (IBGE, 2017). In this context, the minimal of individuals to be sample should be 384. To this study, we analyzed 602 unrelated individuals, born in Goiás, distributed in 25 municipalities of two regions of the state, central (N = 479) and south (N = 123).

The central municipalities of Goiás are Abadiania, Anápolis, Aparecida de Goiânia, Aragoaiana, Beia Vista, Bonfinopolis, Brazarantes, Caldasazinha, Goiânia, Goianira, Itaparanga, Leopoldo de Bulhões, Norpoles, Ouro Verde, Roselandia, Santa Terezinha, Santo Antonio de Goiás, Senador Canedo, Trindade, and Turvania. The south municipalities are Itumbiara, Montividiu, Piracanjuba, Rio Verde, Silvania. The criteria for inclusion in the survey were only those individuals born and live in the State of Goiás. All the municipalities presented tomato, corn, sorghum, and soybean crops (the central plants of the state of Goiás). We obtained blood samples voluntarily according to the Informed Consent Form (TCLE) Data such as age, sex, and social habits were obtained by a lifestyle questionnaire.

2.2. DNA extraction and samples quantification

We sampled 10 ml of whole blood, stored on ice, and immediately sent it to the Mutagenesis Laboratory at the Federal University of Goiás. Genomic DNA samples were obtained from peripheral blood lymphocytes using the Ilustra Genomic Blood Kit® (GE, USA) extraction kit, according to the manufacturer's protocol. DNA samples were then quantified using the NanoVue Plus® (GE, USA) equipment, following the manufacturer's recommendations. After quantification, DNA samples were diluted to a final concentration of 10 ng/μL. The samples were then stored in a freezer at -20 °C, until use.

2.3. Real-time PCR

We performed the analyses of GSTM1 and GSTT1 genes deletion using the real-time polymerase chain reaction (PCR), with SYBR® (Applied Biosystems®, USA), and we also evaluated the co-amplification of the gene RH92600 as an internal control, Marin et al. (2010) previously suggested the primers and the PCR cycling conditions.

For the PCR reaction, the final volume of 25 μL containing 12.5 μL of master mix, 0.5 μL of magnesium chloride (MgCl2), 2.4 μL of forward and reverse primers of the GSTT1 gene, 3.2 μL of forward and reverse primers of the GSTM1 gene, 4 μL of forward and reverse primers of the RH92600 gene, and 1 μL of DNA at 10 ng/μL. We used the Step One Plus thermal cycler Real-Time PCR Systems (Applied Biosystems®, USA).

2.4. Statistical analysis

The sociodemographic characterization data of the sample group was stored in an Excel 2013 worksheet (Microsoft®), along with the data of the polymorphisms, for further evaluation of Statistica 7 software (StartSoft Inc). We estimated frequencies, genotypes, and haplotypes by direct counting. Differences in allele frequencies of GSTT1 and GSTM1 polymorphisms were calculated using the X² test, with a 95% confidence interval (p < 0.05).

3. Results

All 602 individuals are born and raised in Goiás. 68.6% are males, and 31.4% are females. The sample group presented a mean age of 38 ± 12.3 for males and 36 years ± 12.3 for females. We also verified that 51% of males consume alcohol; in contrast, 66.1% of females do not drink alcohol. Finally, 13.6% and 10.6, men and women, respectively, were smokers (Table 1).

The frequency of the GSTT1 null genotype was 38.2%, and for the municipalities with more than 20 individuals sampled (Table 2), it can be seen in Fig. 1A. Therefore, the frequency of the GSTM1 null genotype for

| Sex          | Age (Mean ± SD*) | Alcohol intake | Alcoholic drinker | Non alcoholic drinker | Smoke habit | Non smoker | Smoker | Total (602) |
|--------------|------------------|----------------|------------------|----------------------|-------------|------------|--------|-------------|
| Men (413)    | 38 ± 12.3        | 51% (211)      | 49% (202)        | 86.4% (357)          | 13.6% (56)  | 12.3% (37) | 12.3%  | 37 ± 13.2   |
| Women (189)  | 36 ± 12.3        | 33.9% (64)     | 66.1% (125)      | 89.4% (169)          | 10.6% (20)  | 87.4% (526)| 87.4%  | 36 ± 12.3   |
| Total (602)  |                  |                |                  |                      |             |            |        | 37 ± 13.2   |

* SD = Standard deviation.
In all municipalities of Goias, the frequency of the GSTT1 null genotype was 38.2%, and of the GSTM1 null genotype 50.3%. In the Northeast of India, some authors (Thoudam et al., 2010) demonstrated a frequency of 32.7% for null GSTT1 and 41.9% for the null GSTM1 genotype. Klautau-Guimaraes et al. (2005) evaluated 120 individuals, distributed in two Amerindian tribes of Brazil, the Munduruku tribe of the village of Missão Cururu, and the tribe of Kayabi. The frequency was 27% for null GSTT1 and 0% for null GSTM1 in Munduruku samples. In the Kayabi tribe, those authors detected frequencies of 29% for GSTT1 and 27% for GSTM1 null genotypes. Hiragi et al. (2007) analyzed 91 samples from the Federal District, Brazil, and 273 samples of Brazilian Quilombolas (African offspring) from the states of Bahia, Sergipe, and Goias. Those authors observed frequencies for GSTT1 null genotype in the Federal District of 22% for GSTM1 null genotype of 34% and GSTT1/M1 null genotypes of 11%.

Chiurillo et al. (2013) analyzed 120 urban samples from a Venezuelan population and 188 Amerindian samples, showing a null GSTT1 genotype frequency of 11% and a null GSTM1 genotype of 51% in the urban population. The Amerindian samples were divided into five populations. They presented the following frequencies for the null GSTT1 and GSTM1 null genotypes, respectively: Bari with 11.4% and 54.3%, Panare with 6.5% and 15.2%, Pemon with 0% and 40%, Warao with 0% and 51.7% and Wayuu with 7.9% and 44.7%.

Magno et al. (2009) found lower frequencies for null GSTM1 genotypes (33.2%) and the null GSTT1 genotype (30.2%) in 203 samples from the city of Ilheus, Bahia state, Brazil. Berthou et al. (2001) analyzed 174 samples from Santiago, Chile, and found frequencies for the GSTM1 null genotype of 24%. Rossini et al. (2002) observed frequencies of 42.1% of the GSTM1 null genotype and frequencies of 25.4% of the GSTT1 null genotype in 591 individuals from the state of Rio de Janeiro. However, no significant differences were observed between the genotypes GSTT1 and GSTM1 concerning the population of Goias and the populations of Kalunga (Goias, Brazil), suggesting a genetic similarity between the communities (Table 4).

We observed that the city of Goiania has a different distribution of GSTM1 and GSTT1 frequencies (p < 0.01) regarding all the municipalities of Goias. The frequencies of GSTT1 in municipalities with more than 20 individuals sampled are in Fig. 1B. Finally, the frequencies of GSTT1 and GSTM1 null genotypes (−/−) was 27.24%, −/+ 11%, +/− 23% and ++/++ 39% for Goias (Fig. 1C). Fig. 2 illustrated the haplotypes frequencies in municipalities with more than 20 individuals sampled.

The frequencies for the null GSTT1 were 43.2% (207) in the central region and 18.7% (23) in the south region, and for the null GSTM1 we found frequencies of 54% (259) in the central region and 35.8% (44) in the south region. The distribution of GSTT1 and GSTM1 haplotypes by region was as follows: central region +/- 35.5% (170), -/+ 10.4% (50), -/- 32.8% (157), +/- 21.3% (102) and in the south +/- 51.2% (63), -/+ 13% (16), -/- 5.7% (7), +/- 30% (37) (Table 3).

4. Discussion

Goiania was 50.3%, and for the municipalities with more than 20 individuals sampled are in Fig. 1B. Finally, the frequencies of GSTT1 and GSTM1 null genotypes (−/−) was 27.24%, −/+ 11%, +/− 23% and ++/++ 39% for Goias (Fig. 1C). The frequencies for the null GSTT1 were 43.2% (207) in the central region and 18.7% (23) in the south region, and for the null GSTM1 we found frequencies of 54% (259) in the central region and 35.8% (44) in the south region. The distribution of GSTT1 and GSTM1 haplotypes by region was as follows: central region +/- 35.5% (170), -/+ 10.4% (50), -/- 32.8% (157), +/- 21.3% (102) and in the south +/- 51.2% (63), -/+ 13% (16), -/- 5.7% (7), +/- 30% (37) (Table 3).
municipalities and the other groups and countries (Table 4). Goiania is only like the populations of Riacho de Sucutiaba (Bahia, Brazil) (Hiragi et al., 2007) ($p = 0.891$) and Kayabu (Mato Grosso, Brazil) (Klautau-Guimarães et al., 2005) ($p = 0.103$) (Table 5). Considering the distribution of the null GSTM1 genotype, the other municipalities of our study do not present a significant difference from the Kalunga population and the Federal District, except Goiania. Only the cities of Aparecida de Goiania and Goiania showed substantial differences in the distribution of the null GSTM1 genotype when compared to Rio de Janeiro (Table 6). The municipality of Goiania also differed from all other cities of this study (Aparecida de Goiania, Bela Vista de Goiás, Itapuranga, Montividiu, Silvânia, Turvânia).

The high prevalence of GSTT1 and GSTM1 null genotypes in the municipality of Goiania may reflect a founder effect of non-

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**Fig. 2.** Map illustrating the distribution of GSTT1 and GSTM1 haplotype frequencies in Goias state municipalities with more than 20 individuals sampled.
Singh et al., (2011) found higher values of DNA damage in smokers. Tacca et al., 2019 observed that null GSTT1 and GSTM1 were not associated with the development of cervical cancer in smokers but GSTT1 null genotype was significantly associated with worse prognosis of this disease. In another study conducted in Brazil (Kubiszski et al., 2015), there was a higher prevalence of GSTT1 null with the onset of endometriosis. Singh et al., (2011) found higher values of DNA damage in workers with GSTT1 null exposed to organophosphates.

Table 3

| Region | GSTT1 | GSTT1 + | GSTM1 | GSTM1 + |
|--------|-------|---------|-------|---------|
| Central | 43.2% (207) | 56.8% (272) | 54% (259) | 46% (220) |
| South | 18.7% (23) | 81.3% (100) | 35.8% (44) | 64.3% (79) |

+: Genotype GSTT1 wild type and genotype GSTM1 wild type. -: Genotype GSTT1 null and genotype GSTM1 null.

Table 4

| Country | References | p-value* | p-value ** |
|---------|------------|----------|-----------|
| Mexican | (Mejia-Sanchez et al., 2017) | < 0.0001 | 0.5838 |
| North of Indian | (Thoudam et al., 2010) | 0.08172 | 0.009758 |
| Rio das Rias (Bahi, Brazil) | (Iriagii et al., 2007) | 0.2333 < 0.0001 |
| Mocamb (Sergipe, Brazil) | (Iriagii et al., 2007) | 0.3621 0.08033 |
| Kalunga (Goias, Brazil) | (Iriagii et al., 2007) | 0.1281 0.07792 |
| Distrito Federal (Bahi, Brazil) | (Iriagii et al., 2007) | 0.01328 0.02819 |
| Kayabu (Mato Grosso, Brazil) | (Klaatu-Guimaraes et al., 2005) | < 0.0001 0.07798 |
| Munduruku (Pará, Brazil) | (Klaatu-Guimaraes et al., 2005) | 0.5679 < 0.0001 |
| Venezuela | (Chiuriolo et al., 2013) | < 0.0001 0.9998 |
| Bari (Venezuela) | (Chiuriolo et al., 2013) | 0.002576 0.7783 |
| Panama (Venezuela) | (Chiuriolo et al., 2013) | < 0.0001 < 0.0001 |
| Peron (Venezuela) | (Chiuriolo et al., 2013) | < 0.0001 0.2703 |
| Warao (Venezuela) | (Chiuriolo et al., 2013) | < 0.0001 1 |
| Wayuu (Venezuela) | (Chiuriolo et al., 2013) | 0.000328 0.6158 |
| Ilheus (Bahi, Brazil) | (Magno et al., 2009) | 0.04471 < 0.0001 |
| Rio de Janeiro (Brazil) | (Rossini et al., 2002) | < 0.0001 0.005401 |

Table 5

| Pairwise comparison of GSTT1 frequencies between our population and other authors worldwide. |
|--------|--------|--------|--------|--------|--------|
| APA | BEL | GYN | ITA | MON | SIL | TUR |
| MEX | 0.000 | 0.000 | 0.253 | 0.000 | 0.000 | 0.000 |
| NEI | 0.961 | 0.086 | 0.000 | 0.674 | 0.066 | 0.035 | 0.081 |
| RSB | 0.100 | 0.008 | 0.025 | 0.096 | 0.005 | 0.003 | 0.010 |
| RBB | 0.083 | 0.608 | 0.000 | 0.724 | 0.783 | 0.481 | 0.427 |
| MSE | 0.145 | 0.011 | 0.001 | 0.140 | 0.007 | 0.004 | 0.014 |
| KGO | 0.043 | 0.004 | 0.001 | 0.066 | 0.001 | 0.001 | 0.006 |
| DIS | 0.166 | 0.541 | 0.000 | 0.846 | 0.688 | 0.420 | 0.384 |
| KYM | 0.000 | 0.000 | 1 | 0.000 | 0.000 | 0.000 | 0.000 |
| MUP | 1 | 0.104 | 0.000 | 0.641 | 0.099 | 0.053 | 0.090 |
| VEN | 0.000 | 0.479 | 0.000 | 0.025 | 0.148 | 0.439 | 1 |
| BAV | 0.017 | 0.733 | 0.000 | 0.152 | 0.428 | 0.723 | 1 |
| PA | 0.001 | 0.250 | 0.000 | 0.021 | 0.091 | 0.227 | 0.650 |
| PEV | 0.000 | 0.021 | 0.000 | 0.001 | 0.007 | 0.020 | 0.084 |
| WAV | 0.001 | 0.056 | 0.000 | 0.006 | 0.024 | 0.053 | 0.160 |
| WUV | 0.003 | 0.399 | 0.000 | 0.052 | 0.182 | 0.376 | 0.833 |
| ILB | 0.574 | 0.172 | 0.000 | 0.939 | 0.173 | 0.091 | 0.141 |
| RIO | 0.053 | 0.372 | 0.000 | 0.902 | 0.446 | 0.241 | 0.273 |
| APA | 1 | 0.092 | 0.000 | 0.653 | 0.077 | 0.041 | 0.083 |
| BEL | 0.092 | 1 | 0.000 | 0.431 | 0.943 | 1 | 0.095 |
| GYN | 0.000 | 0.000 | 1 | 0.000 | 0.000 | 0.000 | 1 |
| ITA | 0.653 | 0.431 | 0.000 | 1 | 0.531 | 0.337 | 0.313 |
| MON | 0.077 | 0.943 | 0.000 | 0.531 | 1 | 0.846 | 0.676 |
| SIL | 0.041 | 1 | 0.000 | 0.337 | 0.846 | 1 | 0.965 |
| TUR | 0.083 | 0.959 | 0.000 | 0.313 | 0.676 | 0.965 | 1 |

The central and south regions of Goiás showed frequencies of 43.2% and 18.7% for GSTT1 null and 54% and 35.8% for GSTM1 null (Fig. 3A). These regions together are responsible for the production of 18,356,659 tons of grain in the state of Goiás, which comprises 80.46% of the state's total production (Instituto Mauro Borges, 2018). As Goiás is the fourth-largest producer of grains and is also the fourth-largest user of pesticides (Instituto Mauro Borges, 2018), the null genotypes represent a risk to the population due to high agricultural production and the high handling of pesticides in the state. The mean population age (37 ± 13.2) may represent the basal exposure to pesticides of the people of Goiás. However, another important fact is the number of grains produced by the state; 22,814,803 tons (Instituto Mauro Borges, 2018) can be representative of the number of rural workers (95,745 by 2017) (Instituto Mauro Borges, 2018). For those who have direct contact with the pesticide, the null genotypes represent an even higher risk. In this case, the south region is the most representative region for grain production (17,572,323 tons) (Fig. 3B).

Exposure to pesticides can produce abnormal amounts of free radicals, leading to overproduction of oxidants or an inadequate supply of antioxidants (Corsini et al., 2013). In this context, occupational exposure to pesticides increases DNA damage, as Franco et al. (2016) noted in their study of public state agents who handle pesticides to combat the mosquito Aedes aegypti, which is responsible for the transmission of arboviruses. Those authors observed that recently intoxicated agents had an increased frequency of translocation between chromosomes 14 and 18.

In addition, Roulland et al. (2004) concluded that the BCL2-IGH translocation (14:18) is linked to non-Hodgkin's lymphoma, since it may be a measure of instability caused by the genotoxic effect of the pesticide in a relevant lymphomagenesis. Ventura et al. (2019) demonstrated that 100-day exposure to chlorpyrifos promoted the development of mammary tumors in addition to reliving a hypo-expression of stromal cell receptors.
Zeng et al., 2017). It has also become essential to determine if people used show a higher prevalence and risk of cancer and, ultimately, to living in areas of high agricultural intensity where pesticides are widely used. However, the most recognized factor is the risk of developing specific cancers (Blair and Freeman, 2009; Bolognesi et al., 2011; Stojanovic et al., 2018; Godoy et al., 2019).

Neurological, reproductive, and developmental disorders have already been associated with occupational exposure to pesticides; however, the most recognized factor is the risk of developing specific cancers (Blair and Freeman, 2009; Bolognesi et al., 2011; Stojanovic et al., 2018; Zeng et al., 2017). It has also become essential to determine if people living in areas of high agricultural intensity where pesticides are widely used show a higher prevalence and risk of cancer and, ultimately, to elucidate the mechanisms by which these environmental contaminants relate to the emergence of disease (Alavanja et al., 2013; Parrón et al., 2014). Additionally, along with daily pesticide exposures, people living in areas of intense agricultural activity may interact with other products to which the general population is exposed, such as pollution, dyes, preservatives, and various products that, for example, can affect the immune system, where malfunction leads to the susceptibility of multiple diseases. Genetic factors, such as the presence of polymorphisms capable of increasing the risk of cancer, should be added to the relevant aspects.

In general, the increasing trend in cancer incidence in recent years can be attributed not only to aging but also to the spread of carcinogenic agents that lead to daily exposure. This makes it very important to study the factors related to the risks to which different populations are exposed (Tebourbi et al., 2011).

In Goias, the use of pesticides has grown considerably, with 43,466 kg consumed in 2017 alone (IBAMA, 2019). In this context, knowing the critical role of GST enzymes for detoxification pathways and that haplotypes with null alleles are of high risk for the population, this use reached 61% of the Goias population. Finally, our results indicated that it is essential to know the frequencies of the GSTT1 and GSTM1 genes for the monitoring of risk groups; for example, farmers who have contact with pesticides directly or indirectly, as well as assisting in the development of preventive medicine practices.

5. Conclusions

The use of glyphosate, 2,4-D, acephate, and other pesticides totaling 43,663 tons is related to the high agricultural productivity of Goias, leaving Goias in four places in underreporting numbers of intoxication in Brazil. However, with the new Brazilian government authorizing the use of banned pesticides worldwide, this situation may increase the number of poisonings, especially in Brazilian regions involved with agriculture.

With the high frequency of null genotypes in the south and central regions of Goias, and also with these regions being the first and third in the ranking of grain production respectively, it is of great importance that the government take measures to prevent cases of intoxication, given the risk that the null GSTT1 and GSTM1 genotypes offer for people exposed to pesticides.

In this context, GSTT1 and GSTM1 genotypes may be essential biomarkers of susceptibility to poisoning, mainly in populations with pesticide contact, due to the role of GST enzymes. However, it is necessary to associate GSTT1 and GSTM1 genotyping with other biomarkers such as comet assay, micronucleus test, oxidative stress enzyme measurements, for confirmation, quantification of biological damage and better elucidation of a given natural process.

This study presented a combined frequency (haplotypes) of GSTT1 and GSTM1 genes in Central Brazil. Future studies to monitor the toxicity of occupationally exposed workers and planting areas in which pesticides are used, as well as non-target animals, are necessary to verify human and environmental health, against the use of pesticides in this Brazilian

| MEX | APA | BEL | GYN | ITA | MON | SIL | TUR |
|-----|-----|-----|-----|-----|-----|-----|-----|
| 0.009 | 0.755 | 0.000 | 0.698 | 0.198 | 0.190 | 1   |
| 0.050 | 1   | 0.000 | 0.278 | 0.524 | 0.474 | 0.734 |
| 0.000 | 0.000 | 0.891 | 0.007 | 0.000 | 0.000 | 0.007 |
| 0.191 | 0.063 | 0.000 | 0.003 | 0.140 | 0.233 | 0.047 |
| 0.060 | 0.086 | 0.030 | 0.416 | 0.007 | 0.008 | 0.297 |
| 0.514 | 0.809 | 0.000 | 0.224 | 1   | 0.909 | 0.573 |
| 0.542 | 0.717 | 0.000 | 0.165 | 1   | 0.967 | 0.498 |
| 0.000 | 0.075 | 0.103 | 0.350 | 0.007 | 0.008 | 0.253 |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.003 | 0.523 | 0.000 | 0.988 | 0.101 | 0.101 | 0.971 |
| 0.027 | 0.473 | 0.000 | 1   | 0.145 | 0.137 | 0.831 |
| 0.033 | 0.012 | 0.000 | 0.001 | 0.025 | 0.045 | 0.009 |
| 0.494 | 0.988 | 0.000 | 0.376 | 0.900 | 0.825 | 0.734 |
| 0.078 | 0.648 | 0.003 | 1   | 0.262 | 0.243 | 1   |
| 0.226 | 1   | 0.000 | 0.546 | 0.560 | 0.512 | 1   |
| 0.347 | 1   | 0.000 | 0.280 | 0.496 | 0.450 | 0.744 |
| 0.342 | 1   | 0.000 | 0.000 | 0.549 | 0.705 | 0.646 | 0.919 |
| 0.000 | 0.000 | 1   | 0.002 | 0.000 | 0.000 | 0.002 |
| 0.038 | 0.549 | 0.002 | 1   | 0.183 | 0.171 | 0.917 |
| 0.694 | 0.705 | 0.000 | 0.183 | 1   | 1   | 0.497 |
| 0.862 | 0.646 | 0.000 | 0.171 | 1   | 1   | 0.458 |
| 0.233 | 0.919 | 0.002 | 0.917 | 0.497 | 0.458 | 1   |

Table 6. Pairwise comparison of GSTM1 frequencies between our population and other authors worldwide.

**Fig. 3.** A. Distribution of GSTT1 and GSTM1 in central and south regions of Goias state. B. Amount of grain production in the five regions (central, east, northwest, north and south) of Goias state.
region, which is characterized by intensive agricultural activity.

Declarations

Author contribution statement

Alessandro Arruda Alves: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fernanda Craveiro Franco: Performed the experiments; Analyzed and interpreted the data.

Fernanda Ribeiro Godoy: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Jheniffer Sonora Aguilar Ramos, Hugo Freire Nunes, Thannya Nascimento Soares: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Daniela de Melo e Silva: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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